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THE UTILIZATION OF CARBOHYDRATE BY TOTALLY DEPANCREATIZED DOGS RECEIVING NO INSULIN[1]

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SINCE the pioneer work of Mering and Minkowski (1889), there has accumulated a large amount of evidence that the control which the pancreas exerts on carbohydrate metabolism primarily depends on the production of an internal secretion by certain cells contained in the Islands of Langerhans. The isolation of insulin, by Banting and Best (1922), and the work which has followed it (*cf.* Macleod, 1926), have left little doubt that diabetes mellitus, like the diabetes which results from pancreatectomy in animals, is the result of the lack of available insulin in the animal organism.

From the extensive glycosuria exhibited, and especially from the fact that administered sugar was excreted more or less quantitatively in the urine, Minkowski (1893) concluded that depancreatized dogs have lost the power to utilize carbohydrate. He further observed that, for a varying number of days during an intermediate stage of his experiments, the amount of glucose excreted by the animals bore a fairly constant relationship to the amount of nitrogen excreted in the urine. This relationship, named the D:N ratio, was not affected by varying the amount of protein in the diet and, during those experimental days which Minkowski considered significant, had an average value of 2.8:1. These observations he interpreted to mean that protein was the sole source of the excreted glucose, and that a constant proportion of the protein was being converted into glucose.

Since the work of Minkowski the investigation of the respiratory exchange has led to the conception of the respiratory quotient, the level of which in normal animals has been found to be determined by the relative proportions of fat, protein and carbohydrate being metabolized. The low

[1] Based on a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

R.Q.'s usually exhibited by the diabetic organism led to the conclusion that, in this state, energy is chiefly derived from the oxidation of fat. The negligible effect of the ingestion of carbohydrate on the R.Q. of such animals seemed complete verification of Minkowski's belief that the diabetic organism is unable to utilize carbohydrate.

An alternative theory of the mechanism of diabetes, which has found most support in Europe (*cf.* Geelmuyden, 1923 and Von Noorden, 1927), ascribes the hyperglycemia and glycosuria which occur to an overproduction of glucose from protein and fat. This overproduction, whose chief site is probably the liver, is great enough to cause the accumulation and excretion of excess sugar, in spite of the continued ability of the animal to oxidize it. The evidence which has been summoned in support of this theory is chiefly of two kinds:

1. Evidence for the conversion of fat into carbohydrate in the animal organism. (The occurrence of this process in plants is not disputed (Leathes and Raper, 1925)).

2. Evidence for the oxidation of carbohydrate by the diabetic animal, or by isolated tissues of such animals.

The Conversion of Fat into Carbohydrate
(*Gluconeogenesis from fat*)

If it can be demonstrated that sugar may be derived from fat in the diabetic organism (referring to the fatty acid portion of fat, in particular), the glucose arising from this source may so abundantly supply the tissue requirements that any extra glucose arising from protein will be excreted. It will thus be possible, when the rate of overproduction is more or less constant, to obtain fairly constant average D:N ratios. The level of this ratio, however, cannot then be taken as indicating the proportion of protein which is being converted into glucose, for this level will depend on the relative rates of overproduction and utilization. Similarly, the quantitative excretion of administered glucose may indicate only that the relative rates of overproduction and utilization have not changed during the experiment.

It is not proposed to present here a review of the evidence for gluconeogenesis from fat. Macleod (1928) has recently written a comprehensive monograph on the subject in which he concludes that this process must occur. The recent work of Chaikoff and Weber (1928), however, deserves separate mention as strong support for this conclusion. By repeated administration of epinephrin to totally depancreatized dogs deprived of food and insulin, these workers were able to recover in the urine of such animals

more glucose than could be accounted for by the maximal possible carbohydrate stores in the liver, the breakdown of protein as judged by the nitrogen excretion, and the glycerol portion of that amount of fat which would supply the total caloric expenditure of the animal. This result was obtained even when it was assumed, for the sake of argument, that all the carbon of the protein might be converted to glucose. In some experiments there was extra glucose after allowing for the sugar which might have come from the muscle stores, an eventuality which, in the light of work by Best, Hoet and Marks (1926), and Soskin (1927), is a very unlikely one.

The Utilization of Carbohydrate

If the oxidation of glucose by the diabetic organism can be shown to occur, then the quantitative recovery of administered glucose in the urine must clearly be due to the fact that the organism is already producing more sugar than it can possibly utilize, so that any additional glucose is simply excreted. Sugar arising from protein would suffer a similar fate so that it would be possible, with the reservations mentioned above, to have a constant D:N ratio.

The results of many types of experiment have been interpreted to mean the oxidation of carbohydrate by the diabetic organism. All clinical observations of this nature are subject to the general criticism that it is impossible to tell whether the subject is completely diabetic, or how much insulin the remaining islet cells may be excreting at any given time. Experiments on the depancreatized animal present the inherent difficulty that it is impossible to determine, for any given period, how much glucose is being formed from other food and storage materials. There is, therefore, no basis for estimating from the difference between sugar formation and sugar excretion, how much sugar is being oxidized. If, however, we accept Minkowski's interpretation of the D:N ratio, a value of this ratio lower than 2.8:1 would mean that the animal is not only utilizing all the sugar which is arising from non-protein sources, but is also utilizing some of the sugar arising from the protein. Such low D:N ratios were reported by Minkowski himself (1893) and, as is pointed out below, several workers have since found that the level of the D:N ratio in depancreatized dogs is not nearly so constant as Minkowski once thought.

Experiments designed to show the utilization of carbohydrate by isolated tissues from the depancreatized animal have yielded somewhat contradictory results. But the work of Mann and Magath (1923), like that of Macleod and Pearce (1913), shows very clearly that, with the liver removed, depancreatized as well as normal animals show a steady fall of

the blood sugar, and require a constant injection of glucose to maintain life. This is in strictest accord with the view that the tissues in diabetic animals constantly drain sugar for their use from the blood stream, although this is so much more difficult to demonstrate when the hyperfunctioning liver is *in situ*. This conclusion also derives strong support from the work of Hill (1922) and of Meyerhof (1924), which leads them to conclude that the energy of muscular contraction is almost exclusively derived from the combustion of carbohydrate.

The opponents of the "overproduction theory" on this continent have based their conclusions chiefly upon observations on animals poisoned with phlorhizin. The assumption that these animals are diabetic, in the sense that they are unable to oxidize glucose, is based on the following observations:

(a) They show a marked glycosuria, and excrete administered glucose quantitatively in the urine.

(b) They show a fixed D:N ratio of 3.65:1, which does not vary with ingested protein.

(c) They show an R.Q. which represents the combustion of fat, and which does not rise when sugar is administered.

The validity of some of these observations, such as the quantitative excretion of administered glucose, has long been called into question (Sansum and Woodyatt, 1916). There is also a great deal of evidence that the phlorhizinized animal is a quite different preparation from the depancreatized one. Thus, the phlorhizinized animal retains its pancreas, and Nash and Benedict (1924) have obtained insulin from the pancreas of dogs after prolonged and maximal phlorhizinization. The blood sugar after phlorhizin is low, and the animal may give signs of hypoglycemia. There is considerable evidence that phlorhizin glycosuria is renal in character. Epstein and Baehr (1916), Deuel, Wilson and Milhorat (1927), and others, have shown that nephrectomy in the phlorhizinized dog (also phlorhizinization of the nephrectomized dog) results in a preparation which is quite normal in regard to its blood-sugar level, its respiratory quotient, and the rise in the R.Q. following the administration of glucose. Thus, if we are to believe that the phlorhizinized animal cannot utilize carbohydrate, we must also make the rather curious assumption that the removal of the kidneys restores the power of its tissues in this regard.

The final blow to the belief that the phlorhizinized animal cannot oxidize carbohydrate has been dealt by Wierzuchowski (1927), and confirmed by Deuel, Wilson and Milhorat (1927). These workers showed that the ingestion of glucose by phlorhizinized dogs results in the retention of glu-

cose, which has an antiketogenic and protein-sparing action, and causes a rise in the R.Q. comparable to that which occurs in the normal dog. In the face of all this evidence, it is difficult to understand the process of reasoning by which ratios, *based on the assumption that the phlorhizinized animal cannot burn glucose,* are used to arrive at the conclusion that only "a small amount of glucose may be oxidized" by the phlorhizinized dog (Lusk, 1928). It is even more difficult to accept the use of such data as a basis for maintaining that the entirely different preparation, the depancreatized animal, is unable to oxidize carbohydrate.

A consideration of the facts presented above had clearly indicated that the "theory of nonoxidation" was quite untenable as a rational explanation of the mechanism of diabetes. The "theory of overproduction with utilization" therefore appeared to be the one which accorded best with the experimental evidence. More or less satisfactory evidence existed for gluconeogenesis from fat, and the chief criticisms raised against the oxidation of carbohydrate could be shown to have no real basis. It seemed desirable, however, to obtain more direct evidence for the utilization of carbohydrate by the totally depancreatized animal. Such evidence is here presented.

Method

Totally depancreatized dogs, completely recovered from the operation, and presenting well-healed and non-infected wounds, were used. The animals were maintained on liberal meat-sugar-raw-pancreas diets and insulin, from the time of the operation to the beginning of the experiment. At this time insulin was discontinued, usually after the administration of tapering doses over a period of two days, and the previous diet was substituted by one composed almost solely of protein. The most practical diet, chiefly on account of its palatability, was found to be raw minced beef and granulated gelatin, although dog 2 (Table I) received some Calcium caseinate[2] as well. Care was taken to see that the meat was free from visible fat. For purposes of the experiment known quantities of dextrose dissolved in distilled water were administered by stomach tube from time to time. This dextrose was given in addition to the regular diet, and careful record was kept of the completeness with which the animal finished each meal.

Throughout the entire length of most of the experiments, the sugar, nitrogen and total ketone excretion in the urine were determined for 24-hour periods. These determinations were made by the methods of Shaffer-

[2] Casec, Mead Johnston and Co.

Hartmann (1920–21), and Kjeldahl and Van Slyke (1917) respectively. At frequent intervals the respiratory quotient was determined in a care-

TABLE I

DOG 2 (TOY DOG) — DEPANCREATIZED NOV. 15, 1927

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R. Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.	C.C. per kgm. per hr.	C.C. per kgm. per hr.		Gms.	Gms.		Mgms.	
1	3.65	744	548	.733					Insulin withdrawn. Nov. 25, 1927.
2									
3	3.47	674	461	.685					Protein feeding started Nov. 26, 1927.
4									
5									
6	3.15	659	467	.709					Casec, 4 gms. per Kgm. (Twice Daily)
7									Gelatin, 5 gms. per Kgm. (Twice Daily)
8	3.10	665	483	.728					Meat, 5 gms. per Kgm. (Twice Daily)
9									*Note*—This dog ate only 1/2–2/3 of each meal, as a rule.
10	2.90	583	416	.713					
11									
12					8.04	4.64	1.73	386	
13	2.60	508	434	.854	7.81	3.96	1.97	309	
14	2.60	496	475	.957	6.48	4.15	1.56	113	
15	2.56	544	490	.901	4.95	3.92	1.26	211	
16					3.70	4.88	0.76	161	
17	2.40	678	470	.693	5.00	5.35	0.94	184	
18	2.38	547	502	.917					Insulin started Dec. 13, 1927.
19									
20									
21									
22									
23									Insulin withdrawn Dec.
24					18.50	2.89	6.40	208	18, 1927.
25	2.50	666	467	.701	11.11	2.37	4.70	956	
26	2.35	685	467	.683					This dog was now placed on a meat-sugar-pancreas diet, and was later used for another expt. (Table II).

fully controlled respiratory cabinet of the Benedict type. The R.Q.'s recorded are the averages of 2, 3 or 4 hours, determined in hourly periods. In some of the experiments the CO_2 combining power or the total CO_2 content of the blood was determined by the method of Van Slyke and Neill

(1924) on samples of arterial blood drawn directly from the femoral artery. At termination of some experiments the fat content of the liver was determined by the method of Leathes and Raper (1925). In every case but one (dog 4, Table III) a careful post-mortem examination was made for the presence of residual pancreatic tissue. In some cases the complete absence of islet tissue was verified by microscopic examination. Dogs 1, 4 and 14

TABLE II
DOG 2 (SAME AS IN TABLE I)

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R. Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.	C.C. per kgm. per hr.	C.C. per kgm. per hr.		Gms.	Gms.		Mgms.	
									Insulin withdrawn Jan. 17, 1928.
1	3.90				17.66	3.42	5.16	0	Protein feeding started Jan 18, 1928.
2					15.00	3.02	4.97	961	*Note*—After first few
3	3.20	732	497	.678	10.67	3.12	3.42	3070	meals, the animal
4	3.00	745	507	.680	7.45	2.93	2.54	2202	would eat nothing.
5					4.45	2.54	1.75	873	Thereafter conducted
6					2.97	2.36	1.26	320	as a starvation experi-
7	2.60				2.73	2.33	1.17	265	ment.
8	2.45	762	531	.697	3.57	1.97	1.81	247	Ate 100 gms. lean meat.
9	2.32	799	544	.681	1.91	1.31	1.46	153	
		661	459	.695					*40 gms. cane sugar per os* after preliminary
10	2.15	625	423	.678					observation period.
									Died during night.
									Post-Mortem.
									No pancreatic fragments. Liver very friable and fatty.

(Tables I, III and IX), after giving the results sought by the experiment, were recovered on laboratory diet and insulin, in order to show that the results obtained were not of a "pre-mortal" nature. Dogs 1 and 14 were subsequently sacrificed and post-mortemed. Dog 4 was used for other work.

RESULTS

The results are presented in tabular form below. Although some animals survived longer, and showed more effects than others, no completely negative experiments occurred. The animals showed individual variations in

TABLE III

Dog 4 (TERRIER) — DEPANCREATIZED (DATE UNRECORDED)

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R. Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.	C.C. per kgm. per hr.	C.C. per kgm. per hr.		Gms.	Gms.		Mgms	
									Insulin withdrawn May 21, 1928. Protein feeding started May 21, 1928. viz. 150 gms. meat, twice daily. *Note*—This dog ate all its food throughout the experiment.
1	4.77	588	474	.805					May 22, 1928.
2	4.66	667	461	.692	34.13	9.66	3.53	760	
3	4.66				28.05	9.70	2.89	3040	
4	4.55				27.90	10.25	2.72	5617	
5	4.26								Vomiting. *Gave 5 units Insulin.*
6									Vomiting ceased.
7	4.09	687	517	.754	21.50	8.48	2.53	1445	
8	4.06	702	505	.721	20.13	8.04	2.50	1705	
9	3.98				17.40	7.08	2.46	1375	
10	3.89				20.40	7.53	2.71	1910	
11	3.86	659	469	.711	20.30	7.50	2.71	2120	
12	3.75				16.70	6.86	2.43	1420	
13	3.75				19.40	7.53	2.58	1374	
14	3.66	713	497	.698	21.50	8.06	2.67	1451	
15	3.61	666	468	.702	19.00	7.20	2.64	868	
16	3.52				19.70	7.64	2.58	1178	
17	3.52	676	505	.748	18.00	7.36	2.45	930	
18	3.52	665	483	.728	19.70	8.03	2.45	1030	
19	3.44	719	482	.681	20.10	7.73	2.60	942	
20	3.41				19.90	7.19	2.77	950	
21	3.41	665	496	.745	17.50	7.25	2.41	893	
22	3.32	695	511	.735	15.80	6.86	2.30	821	
23	3.32	685	476	.696	17.80	6.94	2.56	604	
24	3.27	683	491	.719	19.50	7.84	2.49	556	
25	3.27	685	489	.715	19.00	7.45	2.55	570	
26	3.21				19.60	7.11	2.76	507	
27	3.10				19.70	7.53	2.62	592	
28	3.10	667	498	.747	16.70	6.94	2.41	665	
29	2.98				23.54	6.62	—	423	*50 gms. dextrose* per os at 8:45 A.M. *i.e.* 1 3/4 hrs. before end of 29th day.
30	3.10	566	483	.738	50.30	5.29	—	136	
31	2.95	794	571	.719	22.20	8.90	2.49	646	
32	2.95	782	564	.722		8.00		565	
33	2.90								Started on meat-sugar-pancreas diet with *Insulin*. Later used for other work.

TABLE IV

DOG 6 (HOUND) DEPANCREATIZED OCT. 15, 1928

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R. Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.	C.C. per kgm. per hr.	C.C. per kgm. per hr.		Gms.	Gms.		Mgms.	
									Insulin withdrawn Oct. 28, 1928. Protein feeding started Oct. 27, 1928. 200 gms. meat } twice 15 gms. gelatin } daily *Note*—This animal ate all its food, throughout the experiment.
1	5.94	949	660	.696	63.49	17.34	3.66	751	Oct. 30, 1928.
2	5.74				41.69	15.97	2.61	1007	
3	5.45				54.74	17.37	3.15	786	
4	5.08	894	652	.730	35.25	15.33	2.30	820	
5	5.08				41.31	16.08	2.57	303	
6	4.94				34.04	15.20	2.24	493	
7	4.69				39.97	17.50	2.28	581	
8	4.63	1107 1038	783 762	.707 .733	69.84	13.10	0.75	152	*50 gms. dextrose per os.* (Retained 20.13 gms.)
9	4.46				39.24	16.20	2.42	159	
10	4.35				21.00	15.93	1.32	121	
11	4.20	874	694	.795	4.50	11.09	0.40	50	
12	4.10				20.10	12.68	1.58	112	
13	4.03				30.25	14.81	2.04	79	
14	3.92				24.12	15.02	1.60	141	
15	3.69	727 775	586 622	.805 .803	46.96	13.19	0	0	*50 gms. dextrose per os.* (Retained 27.16 gms.)
16	3.72				12.22	14.78	0.83	0	
17	3.58				38.22	13.24	0	0	*50 gms. dextrose per os* eight hours before entering respiratory cabinet on day 18. (Retained 24.00 gms.)
18	3.31	484	438	.906					*Post-mortem* No pancreatic fragments. No subcutaneous fat. Liver fat = 1.5%.

their readiness to take food throughout the experiment. Those which continued to eat all their food almost invariably survived for relatively long periods. Of those animals which refused to eat, dog 2 (Table II) was the only one which survived long enough to make observations possi-

TABLE V

DOG 8 (TERRIER) — DEPANCREATIZED NOV. 23, 1928

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R. Q. (Average)	Sugar Excret	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.	C.C. per kgm. per hr.	C.C. per kgm. per hr.		Gms.	Gms.		Mgms.	Insulin withdrawn Dec. 3, 1928. Protein feeding started Dec. 3, 1928. 200 gms. meat } twice 10 gms. gelatin } daily *Note*—This dog ate all its food, throughout the experiment.
1	5.20	946	660	.697	46.37	14.91	3.11	329	*Dec. 4, 1928.*
2	5.18				42.02	13.74	3.06	621	
3	5.08				37.70	12.95	2.91	682	
4	4.92	859	598	.696	43.32	13.22	3.28	955	
5	4.92				31.50	11.73	2.68	1259	
6	4.62				31.50	10.40	3.03	1345	
7	4.64				62.10	11.42	1.06	949	*50 gms. dextrose at* 3:00 A.M. *i.e.* eight hours before entering respiratory cabinet on day 8.
8	4.45	708	537	.759	34.87	11.89	2.93	1209	
9	4.45				38.42	13.45	2.86	1002	
10	4.46				42.02	12.75	3.29	730	
11	4.66	714 / 558	546 / 406	.765 / .728	51.74	9.09	0.19	435	SEE FOOTNOTE.
12	4.13				33.00	11.31	2.92	780	
13	4.27				32.01	14.03	2.28	869	
14	3.98				34.50	12.38	2.79	559	
15	4.09	892	651	.729					Accidental death, result of heart puncture. *Post-mortem.* No pancreatic fragments Liver fat = 6.13%.

8:15 A.M. CO_2 combining power = 43.2 Vols %.
8:30 A.M. *50 gms. dextrose per os.*
11 A.M. CO_2 combining power = 45.3 Vols. %.
11:25 A.M.–1:25 P.M. First cabinet period.
2 P.M. CO_2 combining power = 39.3 Vols. %.
2:30 P.M.–4:30 P.M. Second cabinet period.
5 P.M. CO_2 combining power = 43.9 Vols. %.

ble. The rest, six in all, died within a few days as a result of the withdrawal of insulin, and their records are not included in the tables. In dogs 4 and 10 (Tables III and VII) it was necessary to give a small dose of insulin on the fifth day of experiment in order to check the onset of vomiting.

Except for the last two or three days of each experiment the animals remained bright and fairly active, notwithstanding the rapid loss of weight which occurred. Even when the animals showed considerable weakness, as a result of the extreme loss of body tissue, it was usual for them to exhibit excitement and activity about meal times. In some cases sustained barking, as in normal healthy animals, was the rule.

TABLE VI

DOG 9 (COLLIE) DEPANCREATIZED JAN. 8, 1929

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R. Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.	C.C. per kgm. per hr.	C.C. per kgm. per hr.		Gms.	Gms.		Mgms.	Insulin withdrawn Jan. 17, 1929. First protein feeding Jan. 17, 1929, viz: 250 gms. meat } twice 10 gms. gelatin } daily *Note*—This dog left variable amounts of its food uneaten, thruout experiment.
1	8.27	779	545	.700	49.00	16.76	2.92		Jan. 18, 1929.
2	7.73				59.85	23.09	2.59		Urine for days 2 and 3 collected together.
3									
4	7.20				17.10	8.12	2.10	1068	
5	7.05	573	397	.692	14.70	6.24	2.35	895	
6	6.96				28.56	12.06	2.37		
7	6.85				14.90	7.39	2.02		
8	6.63	616	420	.681	16.38	7.15	2.29		
9	6.36				9.10	5.33	1.71	575	
10	6.12				5.50	4.12	1.33		
11	5.96				7.70	4.20	1.83		
12	5.89	608	413	.679	6.10	4.13	1.48		
13	5.68				12.30	6.13	2.01	990	
14	5.86				16.30	8.54	1.91	2303	
15	5.72	611 / 665	423 / 487	.691 / .732	51.25	4.23	0.29	525	SEE FOOTNOTE p. 110.

TABLE VI (*continued*)

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R.Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
16	5.43				8.50	4.66	1.82	784	No respiratory determinations were made after the 15th day because of technical difficulties.
17					8.80	4.87	1.81		
18	5.30				10.20	5.39	1.89		
19	5.17				8.70	5.38	1.62		
20	5.10				8.70	5.24	1.66		
21	4.97				7.80	4.93	1.58	675	
22	4.88				4.80	3.74	1.28	621	
23	4.71				2.00	2.72	0.73		
24	4.55				2.50	2.42	1.03		
25	4.57				3.00	2.80	1.07		
26	4.45				3.10	2.73	1.13	677	
27	4.41								CO_2 content on 27th day = 38.8 Vols. %.
28									Animal found dead on morning of 28th day. *Post-mortem.* No pancreatic fragments. *Confirmed by microscopic examination.* Liver fat = 12.48%.

11:20 A.M.–1:20 P.M.—First cabinet period.
1:40 P.M. CO_2 content = 25.5 Vols. %.
1:50 P.M. *50 gms. dextrose per os.*
2:25–4:25 P.M.—Second cabinet period.
5:50 P.M. CO_2 content = 28.0 Vols. %.
8:40 P.M. CO_2 content = 29.9 Vols. %.
(Retained 15.05 gms. dextrose.)

SUMMARY

The depancreatized dogs used in these experiments differed from most animals used for previous work of a similar nature, in that they were allowed, by the use of insulin, to make a perfect recovery from the operation and anesthetic. These dogs, after the withdrawal of insulin, were kept on a protein diet and generally showed a progressive rise in the respiratory quotient, after the first week or two of the experiment. The R.Q. might be maintained far above the "diabetic" level for as long as two weeks, and in some cases rose above 0.900. At a very early stage of the experiment, the second or third day in some cases, the D:N ratio had usually fallen below 2.8:1 and might be maintained far below this level for almost a month (*e.g.*, dog 9, Table VI).

The administration of 50 gms. of dextrose in addition to the regular diet resulted in the retention of considerable amounts of this sugar, which

was not excreted on subsequent days. This retention of glucose was often as much as 50 per cent of the amount administered, and in one case (dog 14, Table IX) where 100 gms. dextrose was given, the retention actually amounted to 68 gms. In a few instances the total amount of glucose ex-

TABLE VII

Dog 10 (BULL TERRIER) DEPANCREATIZED JAN. 15, 1929

Day of Expt.	Weight				Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.				Gms.	Gms.		Mgms.	
									Insulin withdrawn Jan. 26, 1929.
									Protein feeding started Jan. 26, 1929.
									200 gms. meat } twice 10 gms. gelatin } daily
									Note—This animal ate all its food throughout experiment.
1	4.81				46.84	13.05	3.59		Jan. 28, 1929.
2	4.99				22.80	9.66	2.36		
3	4.70				28.10	9.86	2.85		
4	4.45				21.34	9.10	2.34		Vomiting.
5	4.11								Vomiting. *Gave 3 units Insulin.*
6	4.22				29.10	10.47	2.78		Vomiting ceased. CO_2 content = 49.1 Vols. %.
7					31.46	11.27	2.79		
8	4.10				32.88	11.73	2.80		
9	3.87				28.80	11.04	2.61		CO_2 content = 49.1 Vols. %.
10	3.83				29.90	11.50	2.60	356	
11	3.68				26.60	10.82	2.46	287	
12	3.60				44.10	4.66		0	SEE FOOTNOTE.
13	3.16								Died towards end of 13th day.
									Post-mortem.
									No pancreatic fragments.
									Liver fat = 5.36%.

10:25 A.M. CO_2 content = 41.5 Vols. %.
11:00 A.M. *25 gms. dextrose per os.*
11:30 A.M. *25 gms. dextrose per os.*
12:30 P.M. CO_2 content = 47.3 Vols. %.
1:30 P.M. CO_2 content = 48.3 Vols. %.
3:45 P.M. CO_2 content = 47.8 Vols. %.
6:30 P.M. CO_2 content = 47.5 Vols. %.
(32.50 gms. dextrose retained)

creted on the day of dextrose administration was less than that given, so that even if it could be imagined that on those days the animal produced no glucose from other sources, there would still be a retention. The dextrose administration often caused a decided rise in the R.Q. The progres-

TABLE VIII

DOG 12 (AIREDALE) — DEPANCREATIZED FEB. 12, 1929

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R. Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.	C.C. per kgm. per hr.	C.C. per kgm. per hr.		Gms.	Gms.		Mgms.	Insulin withdrawn Mar. 3, 1929. Protein feeding started Feb. 28, 1929. 200 gms. meat } twice 10 gms. gelatin } daily *Note*—This dog ate about ½ of each meal offered it.
1									March 4, 1929.
2									
3									
4									
5	3.49	756	568	.752					
6	3.40				6.15	3.25	1.89	291	
7					5.35	3.28	1.63	295	
8	3.17				1.00	1.86	0.54	140	
9	3.03	606	556	.917	0.38	1.54	0.25	50	Nasal discharge. Very weak.
10	2.86								Died. *Post-mortem.* Bronchopneumonia. No pancreatic fragments. Microscopic examination revealed three microscopic fragments, but containing no islet cells.

sive increase in this effect, as the experiment advanced, is beautifully illustrated in the results of Table IX. The adminstration of dextrose also caused a sharp drop in the total ketone excretion, and usually exerted a definite protein-sparing action.

When the CO_2 combining power or total CO_2 content of the blood was determined at intervals, very little fall in these values was observed to

TABLE IX

Dog 14 (MASTIFF) DEPANCREATIZED MARCH 20, 1929

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R. Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.	C.C. per kgm. per hr.	C.C. per kgm. per hr.		Gms.	Gms.		Mgms.	Insulin withdrawn Mar. 31, 1929. Protein feeding started March 31, 1929, viz: 250 gms. meat } twice 15 gms. gelatin } daily *Note*—This dog ate all its food throughout the experiment.
1									April 1, 1929.
2	12.21	722	474	.657	85.17	24.25	3.51		
3	12.21				85.28	27.13	3.14		
4	11.94				79.30	25.72	3.08		
5	11.66	733	502	.686	90.93	27.35	3.32		CO_2 content = 36.0 Vols. %.
6	11.44								
7									
8	11.11				76.13	22.93	3.32		
9	10.53	734 / 701	490 / 497	.668 / .709	134.55	23.96	1.44		SEE FOOTNOTE 1 p. 114.
10	9.90				71.83	24.89	2.89		
11	10.24				84.00	26.58	3.16		
12	9.94	807 / 744	557 / 537	.690 / .722	157.45	23.73	2.42		SEE FOOTNOTE 2 p. 114.
13	9.51				77.82	23.63	3.29		
14	9.51				69.42	23.39	2.97		
15	9.11								
16	8.60	561	393	.700					
17	8.73				72.83	23.92	3.04		
18	8.40				77.13	25.15	3.07		
19	8.25	814 / 769	559 / 577	.687 / .750	157.20	22.58	2.53		SEE FOOTNOTE 3 p. 114.
20	8.25				82.59	25.08	3.29		
21	7.88				57.38	21.29	2.70		
22	7.87				46.81	20.95	2.23		
23	7.39	723 / 675	516 / 549	.714 / .813	78.18	16.32	0		SEE FOOTNOTE 4 p. 114.
24	7.03				32.30	20.54	1.57		CO_2 content = 40.4 Vols. %

TABLE IX (*continued*)

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R. Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
25	6.84				14.41	20.35	0.71		
26	6.82	726	564	.776					Started on meat-sugar pancreas diet, with Insulin after this respiratory determination. (April 26, 1929)
42									Insulin withdrawn May 12, 1929.
43	8.27								
44	8.15	851	585	.687					

1. 9:21 A.M.–11:21 A.M. First Cabinet period.
   11:30 A.M. *50 gms. dextrose per os.*
   1:00 P.M. *50 gms. dextrose per os.*
   1:14 P.M.–4:14 P.M. Second cabinet period.
   (Retained 41.58 gms.)
2. 9:44 A.M.–11:44 A.M. First cabinet period.
   11:45 A.M. *50 gms. dextrose per os.*
   1:15 P.M. *50 gms. dextrose per os.*
   1:36 P.M.–4:36 P.M. Second cabinet period.
   (Retained 26.55 gms.)
3. 9:34 A.M.–11:34 A.M. First cabinet period.
   11:45 A.M. *50 gms. dextrose per os.*
   1:15 P.M. *50 gms. dextrose per os.*
   1:45 P.M.–4:45 P.M. Second cabinet period.
   (Retained 19.93 gms.)
4. 10:40 A.M.–11:40 A.M. First cabinet period.
   11:45 A.M. *50 gms. dextrose per os.*
   1:15 P.M. *50 gms. dextrose per os.*
   1:45 P.M.–4:45 P.M. Second cabinet period.
   (Retained 68.63 gms.)

occur throughout the experiment. Dogs 9 and 14 (Tables VI and IX) actually show a rise in these values during the later stages of the observation. The administration of dextrose invariably caused a temporary retention of CO_2. It is evident, therefore, that the "blowing-off" of CO_2 was not a factor influencing the R.Q. The retention of CO_2 associated with the diminished ketone excretion probably accounts, as Wierzuchowski (1926) has suggested, for those cases in which the administration of dextrose had little effect on the R.Q.

The fat content of the liver, where determined, was found to be very much lower than the values reported by Macleod (1926) for diabetic dogs

deprived of sugar and insulin for shorter periods. Thus, dog 6 (Table IV), which demonstrated the oxidation of carbohydrate very clearly, had a liver fat content of only 1.5 per cent at the termination of the experiment.

The three dogs, which were returned to meat-sugar-pancreas diets with insulin, made prompt recoveries. Two of these, which were put back on experiment after a short interval, again gave "diabetic" respiratory quotients.

Discussion

Every criterion, upon which is based the hypothesis that carbohydrate is directly oxidized in the normal animal, has been obtained in the totally

Table X

Dog 15 (Terrier) Depancreatized March 21, 1929

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R.Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
	Kgms.	C.C. per kgm. per hr.	C.C. per kgm. per hr.		Gms.	Gms.		Mgms.	Insulin withdrawn Mar. 31, 1929. Protein feeding started March 31, 1929, viz: 200 gms. meat } twice 10 gms. gelatin } daily *Note*—This dog ate all its food throughout the experiment, except where noted.
1									April 1, 1929.
2	6.40	766	517	.675	40.69	13.62	2.98		
3	6.50				27.22	13.63	1.99		
4	5.98				34.19	13.94	2.45		CO_2 content = 26.9 Vols. %.
5	5.93								Starting to vomit.
6	5.22								*Gave 8 units Insulin.*
7									Vomiting ceased.
8									Ate very little. *Gave 5 units Insulin.*
9									Appetite improving.
10									Ate all its food after this day.
16	4.55	869	593	.682					
24	4.10								CO_2 content = 43.2 Vols. %.
25	4.01								
26	3.92	996	682	.685					
30	3.58	920	648	.704					

TABLE X (*continued*)

Day of Expt.	Weight	Oxygen Intake	CO_2 Output	R.Q. (Average)	Sugar Excret.	Nitrogen Excret.	D:N	Total Ketone Excret.	Remarks
31	3.52				23.43	10.55	2.22		
32	3.47				24.30	10.44	2.33		
33	3.47	886 / 731	612 / 564	.691 / .771	44.85	11.01	0		SEE FOOTNOTE 1.
34	3.31				19.40	10.24	1.89		
35	3.29				11.00	7.85	1.40		
36	3.19				13.86	8.43	1.64		
37	3.06	698 / 537	526 / 458	.754 / .853					SEE FOOTNOTE 2.
38									Died early this morning. *Post-mortem.* No pancreatic fragments.

1. 10:02 A.M.–12:02 P.M.—First cabinet period.
   12:15 P.M.–*25 gms. dextrose per os.*
   1:45 P.M.–*25 gms. dextrose per os.*
   2:05 P.M.–4:35 P.M.—Second cabinet period.
   5:00 P.M.–CO_2 content 44.0 Vols. %.
   (Retained 29.45 gms. dextrose)
2. 10:10 A.M.–12:10 P.M.—First cabinet period.
   12:30 P.M.–*25 gms. dextrose per os.*
   2:00 P.M.–*25 gms. dextrose per os.*
   2:15 P.M.–4:15 P.M. Second cabinet period.

depancreatized dog receiving no insulin. The results are conclusive, and, if we are to continue to regard our criteria as having any real value, we must conclude that the totally diabetic animal suffers little or no impairment in its ability to oxidize carbohydrate. These results are confirmed by isolated observations of other investigators. Minkowski (1893) reported low D:N ratios occurring towards the end of his experiments. He attributed them, however, to the weakness of his animal and interpreted them to mean, not the utilization of carbohydrate, but a decrease in the proportion of protein which was being converted to glucose. Since this interpretation has nothing but a purely speculative basis, there is just as much reason to deny it as to affirm it. In fact, the assumption that protein may give rise to different amounts of sugar at different times, detracts from the significance of his original conclusions regarding the D:N ratio. Other investigators have also obtained D:N ratios below 2.8:1. Their work has been criticized on the grounds of the poor condition of their animals, or incomplete depancreatization. Such criticism, however, can-

not be leveled at the work of Markowitz (1925), who used pancreatectomized dogs maintained in excellent condition by the use of insulin. After the withdrawal of food and insulin from such animals (which by subsequent post-mortem examination were shown to be completely depancreatized), he obtained, after the first few days of the experiment had elapsed, D:N ratios far below 2.8:1. Chaikoff, Macleod, Markowitz and Simpson (1925) reported similar results, and found, moreover, that the D:N ratio was generally higher in fat than in lean dogs, and varied decidedly in the same animal, according to its nutritional condition at the time of the experiment. Hédon (1926) observed R.Q.'s in depancreatized dogs, which were quite comparable to the R.Q.'s obtained from normal animals under similar conditions. Toward the end of his experiments the R.Q. often rose to levels indicating the oxidation of a large proportion of carbohydrate. From a host of clinical observations, it will be sufficient to mention the work of Joslin (1923), who obtained extremely high respiratory quotients in severely diabetic, emaciated subjects.

It might be suggested, for the sake of argument, that the evidence for the direct oxidation of carbohydrate, which appears at later stages in these experiments, is due to the gradual assumption of the faculty of insulin production by glands or tissues other than the pancreas. That this is not the case, however, is clear from the fact that these animals do not live indefinitely, as they do with insulin injections. Nor do severely diabetic humans, without treatment, show permanent, spontaneous improvement of this type. The view that extra-pancreatic sources of insulin may develop becomes quite untenable when it is noted that dogs 1 and 14 (Tables I and IX) which, according to the results obtained, should have acquired a fairly adequate insulin supply, were as "diabetic" as ever when insulin injections were discontinued for the second time. The animals can therefore not be regarded as having been rendered less diabetic in any true sense. The experimental procedure has simply enabled us to maintain the animals in good condition for a sufficiently long time to unmask the process of carbohydrate oxidation, which has been proceeding all the while, without the aid of insulin from the pancreas.

As was pointed out in the introductory remarks, the fact that totally diabetic animals oxidize carbohydrate has led us to adopt the "overproduction theory" of diabetes. From this standpoint the different levels of the D:N ratio observed by the various workers are readily understood. The value obtained, which is constant as long as the particular factors in the animal remain constant, depends on the balance between the production of glucose and its utilization. The simultaneous operation of these two factors also explains the consistently low R.Q. ordinarily obtained in

the diabetic organism. Glucose is being derived from protein and fat, both of which are poorer in oxygen than carbohydrate. The conversion is therefore accompanied by a retention of oxygen. The R.Q. for the conversion of protein to glucose may be regarded as lying between 0.706 (Geelmuyden) and 0.632 (Lusk). The R.Q. for gluconeogenesis from fat has been calculated to be 0.281 (Macleod). The usual diabetic R.Q. of about 0.7, may therefore be regarded as the resultant of two processes proceeding simultaneously—gluconeogenesis (especially from fat) with an R.Q. of 0.2, and the oxidation of carbohydrate with an R.Q. of 1.0 (*cf.* Macleod, 1928).

From the foregoing, it is evident that the results obtained in these experiments must have been due to an increase in the relative amount of carbohydrate being oxidized, or a diminution in the process of gluconeogenesis, or a combination of both. Since the amount of oxidation must be judged from the oxygen intake, which varies with gluconeogenesis as well as with oxidation, it is difficult to estimate how much each of these processes may have varied. A decrease in gluconeogenesis might result from damage to the liver, which, from the work of Best, Hoet and Marks (1926), Bollman, Mann and Magath (1925), and Soskin (1927), is the organ chiefly responsible for this process. It might be suggested that the liver became damaged by toxic metabolites, resulting from the diet composed exclusively of protein. A more probable explanation for decreased gluconeogenesis, however, hinges on the fat stores of the animals. It has been pointed out that the percentage of liver fat, in those experiments where this was determined, was quite low as compared with the liver content in diabetic dogs deprived of insulin for shorter periods. Decreased gluconeogenesis may therefore occur, not because of liver injury, but through a decrease in fat stores, the chief raw material for this process. Gluconeogenesis would then have to proceed at the expense of protein, so that the R.Q. would rise, and the administration of sugar would further raise the quotient because of its protein-sparing action. If this be the case, the return of dogs 1 and 14 (Tables I and IX) to the "diabetic" state after the second withdrawal of insulin, can be explained as being due to the deposition of some fat during the recovery period.

Since the diabetic organism oxidizes carbohydrate, it can no longer be assumed that the absence of this process causes a disturbance in the utilization of fat, whereby the ketone bodies arise. In short, we must find a more exact, if less picturesque, conception than the one commonly quoted as "Fat burns in the fires of carbohydrate, etc."

The work of Chaikoff and Soskin (1928) suggests a more rational view. They found that acetoacetic acid, injected in the form of its sodium salt,

disappeared with equal rapidity from the blood of normal and eviscerated dogs. The acetone bodies, which disappeared from the blood stream, could not be accounted for by those found in the muscle tissue several hours after the injection. This indicated that the muscle tissue was of chief importance in the utilization of these substances. Similar experiments on depancreatized dogs showed a slower disappearance of the injected ketones from the blood stream. When, however, the diabetic dogs were eviscerated the rate of disappearance of ketones from the blood coincided with their rate of disappearance in normal and eviscerated normal animals. The slower disappearance in the intact diabetic animals could therefore not have been due to diminished utilization by the muscles, but must have been due to the fact that the liver was constantly adding ketone bodies to the blood stream, thus diminishing the apparent rate of fall. The conclusion was therefore drawn, that the appearance of ketone bodies in excess amounts in the tissue fluids of diabetic animals is probably due to an overproduction of these substances by the liver. This accords very well with the conclusions drawn from the present work. If the ketones as advocated by Geelmuyden (1923) and Macleod (1926), may be regarded as intermediary products in the conversion of fat to carbohydrate, their accumulation in diabetes (like the accumulation of glucose) is due to the excessive gluconeogenesis which characterizes this condition.

It is evident that a re-interpretation of the meaning of the D:N ratio and the R.Q. is now necessary. We can regard the D:N ratio of 2.8:1, as indicating the proportion of glucose which may be derived from protein, only if we assume that (when this value is obtained) the animal is utilizing just that amount of carbohydrate which is coming from non-protein sources. This is possible but has yet to be proven. The fact that this ratio was not obtained in the present experiments, nor in those of other workers, and the quite different ratio obtained with phlorhizin, would seem to cast considerable doubt on its significance. It is also apparent, that the respiratory quotient cannot be justly considered from the point of view of oxidation alone. The interpretation of the low R.Q. of diabetes has already been discussed. But since the R.Q. obtained from the complete metabolism of protein or fat must ultimately approximate the theoretical R.Q.'s for these materials, no matter what the intermediary processes, it is difficult to explain the R.Q's above 0.8, obtained on an exclusive protein diet. Such R.Q's must depend on intermediary mechanisms, which are as yet little understood. In view of the rapid loss of weight which occurs, Joslin's (1923) suggestion that sugar is being converted into fat, which is stored, is highly improbable. The de-carboxylation of certain of the intermediary products of gluconeogenesis is suggested as a possibility to be considered.

Conclusions

1. As judged by the usually accepted criteria, there is no evidence that the diabetic organism suffers any impairment in its ability to oxidize carbohydrate.

2. The results obtained are incompatible with the "Theory of Non-oxidation," and furnish strong proof for the "Overproduction Theory" of diabetes.

3. The revision of the meanings given to the D:N ratio and the R.Q., which this and other work necessitates, is discussed.

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THE RESPONSE OF THE MONKEY (*MACACUS RHESUS*) TO WITHDRAWAL OF VITAMIN A FROM THE DIET

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THE differentiation, during recent years, of the fat-soluble vitamins A and D (1, 2, 3, 4, 5, 6), the great extension of the knowledge of the factors operating to produce rickets (5, 6, 7) and the discovery of methods of feeding animals a diet containing ample quantities of the antirachitic vitamin without introducing vitamin A (5, 6), have made it possible to study the effects of vitamin A deficiency more accurately than heretofore. Microscopic study of the tissues of rats (8, 9, 10) and guinea pigs (11) dying as a result of absence of vitamin A from the diet, has revealed that certain pathological changes are characteristic of the condition produced in these two species, whose responses to the dietary defect are otherwise dissimilar. Ophthalmia, which is so common a manifestation of vitamin A deficiency in rats (12, 13, 14) and has been observed also in dogs (15), rabbits (16, 17), calves (18), and chickens (19, 20), was not observed in guinea pigs, nor were the gross pathological conditions (tongue abscesses, sinusitis, mastoiditis) so frequently seen in rats (12, 21, 22) detected in guinea pigs. Keratomalacia and hemeralopia in human beings have been attributed to deficiency of the A-factor in the diet (23, 24, 25, 26, 27). Other conditions in animals or man which have been attributed to this cause are diarrhea (13, 18, 23, 24), respiratory disturbances (22, 25, 28), urolithiasis (29, 31), calcification of kidney epithelium (32), edema (24, 33), and gastric carcinomas (34).

The only data on record with regard to the effect of deprivation of fat-soluble vitamins on monkeys are those of Harden and Zilva (33), who fed three monkeys for a preliminary period of 198 days a ration otherwise suitable but lacking in vitamins A and D, and then supplemented the ration of one animal with 10 grams of butterfat daily and of another with 10 grams of olive oil. The animal which received the olive oil died 33 days later, *i.e.*, 262 days from the time it was placed on the experimental diet, but showed no abnormalities at autopsy other than emaciation. The one which received neither oil nor butterfat maintained its weight for a month, lost weight during the succeeding month, finally developed diarrhea and edema, and was killed when moribund on the 289th day of the

experiment. The animal which received butterfat remained well throughout the period of observation, though it did not gain in weight.

McCarrison's studies (35) of the effect of deficient diets on monkeys were carried out with rations which lacked two or more vitamins. He regarded the severe gastro-intestinal disturbances which he observed in 21 of 25 monkeys as the characteristic reaction of the monkey to a diet containing insufficient amounts of the vitamin factors.

The experiment to be reported here was undertaken, at the suggestion of Dr. Hideyo Noguchi, with a view to finding out whether monkeys deprived of vitamin A would show any differences from normal monkeys in their resistance to the infectious eye disease caused by *Bacterium granulosis*. This micro-organism was isolated by Dr. Noguchi in 1926 from the eyes of Indians suffering from trachoma (36), and it produces in monkeys (*Macacus rhesus* and chimpanzees) a chronic granular conjunctivitis closely resembling human trachoma. The development of the infection in inoculated animals does not invariably ensue, however, and the fact that some monkeys showed no response to inoculation suggested the possibility of operation of a factor leading to increased local or general resistance in such animals. Some discussion of malnutrition as a possible factor in the causation of trachoma has appeared in the literature (37).

The question at issue proved difficult of solution, inasmuch as the period of survival of monkeys on a diet deficient in vitamin A proved to be relatively short, while the incubation period of the *granulosis* infection is rather long. The observations on the effects of depletion of vitamin A reserves in the monkeys, however, furnish data which are interesting for comparison with similar data for other animals and for their bearing on the general subject of nutritional disease.

Young animals, 1 to 1½ years old (1,900 to 2,500 grams) were obtained. Because of the frequent occurrence of tuberculosis among *Macacus rhesus*, preliminary roentgenograms were made of the lungs. Among 18 monkeys purchased in the market, and recently imported from India, 8 were found which showed no pulmonary shadows. Four animals had slight, and 6 marked evidence of pulmonary involvement of some sort. The last group of 6 was not used; the other 12 were divided into two groups in such a way that each group would contain 4 monkeys in which the roentgenograms had been clear and 2 in which there were some indications of lung lesions. The animals were x-rayed again after 2 months and 4 months. As will appear later, the shadows found in three instances either were not indicative of lesions, or the lesions disappeared, since the lungs appeared normal at autopsy. In the fourth monkey, which was on the deficient diet, early bronchopneumonic involvement was detected by microscopic study of lung tissue taken at autopsy.

After 82 days, four additional monkeys, older animals, which had been used for other purposes, were placed on the deficient ration, and another was added a month later. All were healthy and apparently free from lung lesions. This latter group of animals had been on the stock diet during their use in other experiments. The original 12, on the other hand, had come directly from the animal dealer, and their diet had been white bread and carrots, with water instead of milk.

The monkeys were kept in cages with wire bottom of large enough mesh to allow feces to fall through. The removable pans below the wire bottoms were cleaned daily and lined with fresh newspapers, and the cages were scrubbed twice weekly with lysol followed by soap and water. Two animals of the same group were kept in a cage, but during the feeding period a heavy wire partition was inserted to separate them.

The Diet

The diet was based, in its fuel value and protein content, on the stock monkey diet employed at the Rockefeller Institute, which consists of 1 banana, 1 thick slice of white bread, and 1/2 pint of milk daily for each monkey. The fuel value of the stock diet is about 460 calories, and the ration furnishes about 15 grams of protein, 8 grams of fat, and 82 grams of carbohydrate. A ration of similar fuel value and protein content, but lacking the vitamin factors, was constructed by replacing the white bread with rice (43.5 grams) and supplementing the rice with egg white (100 grams), a small amount of sucrose (25 grams), and 10 to 15 grams of aerated butterfat. The fat content of this ration was greater and the carbohydrate content correspondingly less than that of the stock diet, but by the use of the aerated butterfat in the A-deficient diet and the fresh butterfat in the control ration, the fat content was a constant factor in the two rations. No indication that fat is a disturbing element in the diet of monkeys is found in the results of the experiment.

The butterfat was freed from water and milk and aerated as described by Hopkins (2), an oil bath being used to maintain a temperature of 120° C. and air from a compressed air line being blown through the liquid fat for 8 hours. The fat was pure white when cool and contained too little vitamin A to be detected by tests in rats (38). The fresh butterfat, on the other hand, contained an abundance of the vitamin, as shown by the fact that normal growth was obtained in rats receiving 100 mg. or more daily in addition to the basal ration.

Vitamins B and G (39) were furnished by feeding 5 grams of dried yeast (Harris) daily, and sufficient mineral elements were assured by the addition of 5 grams of Osborne and Mendel's salt mixture (40). A small amount of sodium chloride (2 grams) was used to make the food more palatable. To supply the antiscorbutic vitamin without introducing appreciable amounts of vitamin A was a problem not easily solved, but the rutabaga turnip, or swede, was selected as furnishing the largest amount of vitamin C and very little vitamin A. The requirement of monkeys for the antiscorbutic vitamin had been found by Harden and Zilva (41) to be the same as for the guinea pig, a daily dose of 2 cc. of orange juice proving sufficient to protect a monkey against scurvy. Chick and Rhodes

TABLE I

	First period (84 days)					Second period (84th to 111th day)					Third period (after 111th day)				
	Weight gms.	Protein gms.	Fat gms.	Carbohydrate gms.	Fuel value cal.	Weight gms.	Protein gms.	Fat gms.	Carbohydrate gms.	Fuel value cal.	Weight gms.	Protein gms.	Fat gms.	Carbohydrate gms.	Fuel value cal.
Rice	43.5	3.48	0.13	34.4	150	58–65	4.64–5.2	.174–.195	45.82–51.35	200–225	87	6.96	.261	68.73	300
Egg white	100	12.3	0.2		51	100	12.3	.2		51	100	12.3	.2		51
Butter-fat*	10–15		10–15		90–135	15		15		135	15		15		135
Sucrose	25			25	100	25			25	100					
Salt mixture	5					5					5				
NaCl	2					2					2				
Yeast	5	?	?	?		5	?	?	?		5	?	?	?	
Rutabaga (Swede)	50	0.45	0.05	3	14	50	0.45	0.05	3	14	25	.225	.025	1.5	7
Totals		16.	10.4–15.4	62	405–450		17.4–18.	15.4	74–79	500–525		19.5	15.5	70	493

* Fresh for the control group; aerated for 8 hrs at 120° C. for the experimental group.

(42) and Delf (43) had found that raw swede juice in a dose of 5 cc. was ample to protect a guinea pig against scurvy, and Steenbock and Gross (44) had shown that the swede contained a negligible amount of vitamin A. Fifty grams of raw swede yield about 15 to 18 cc. of juice, *i.e.*, about three times the minimum requirement of antiscorbutic vitamin for the guinea pig. This dose was used for 111 days but was reduced by one-half in the latter part of the experiment, with no ill effects which could be detected in the control animals. The vitamin A content of the swede was determined in rats (38) to be about one unit to 4 grams. The A-deficient diet contained, therefore, during the first 111 days, 12 units of vitamin A, *per diem*, and thereafter 6 units.

A daily dose of 50 grams of swede was found by Mellanby and Killick (45) to protect rabbits against rickets. Moreover, the butterfat, even after 8 hours aeration, probably contained some vitamin D. Nevertheless, the animals were exposed twice a week for half an hour to the light of a 65 volt Cooper Hewitt mercury arc lamp, their eyes being protected by headcaps made of two thicknesses of heavy black sateen. The distance was approximately 3 feet, though variations were inevitable because of the movements of the animals.

The monkeys became accustomed to the new ration within a week and ate practically all of it. The proportion of butterfat was increased after 28 days from 10 to 15 grams. After 84 days the amount of rice was increased to 58 grams. The food mixture was still completely consumed, however, and a further increase to 65 grams was made a week later. Finally owing to the prevalence of diarrhea among the animals on the deficient ration, the amount of turnip was reduced one-half, the sucrose was omitted and the rice was increased to 87 grams. The constituents of the diet are recorded in the table (Table I). The estimates of nutritive values were obtained from various sources (46, 47, 48).

Rice sufficient for all the monkeys was cooked on the steam bath in distilled water (in proportion of 250 cc. to 100 grams rice) previously brought to boiling over a free flame and containing the sodium chloride. After one to one and one-half hours cooking the fresh egg whites and the sucrose and salt mixture were added, with thorough mixing, and the whole was cooked another half hour, or until it was rather dry. It was then distributed into individual dishes, and while it was still warm weighed portions of butterfat and yeast were folded into the top layer. On Saturdays two lots of food were prepared, and one was kept in the refrigerator (4°C.) until used on Sunday.

The basal ration (rice, egg white, sucrose, salts, and yeast) was shown by rat tests to be a satisfactory basal ration for quantitative estimation of vitamin A, comparing favorably with the Sherman and Munsell ration (38).

Effect on Monkeys of the Diet Deficient in Vitamin A

Effect on growth

The monkeys were weighed every two weeks. The weight curves are shown in Charts 1, 2, and 3. Charts 1 and 2 are the records of the original twelve young normal animals, six of which (Chart 1) were on the control ration, and six (Chart 2) on the deficient ration. The five animals of Chart 3 were a heterogeneous group which had been used for other purposes; they were placed on the deficient diet three months after the beginning of the experiment, just before the original group on this ration had begun to manifest the first signs of illness, and a month before the amount of the vitamin A in the deficient ration was reduced from 12 units to 6 by reduction in the dose of swede.

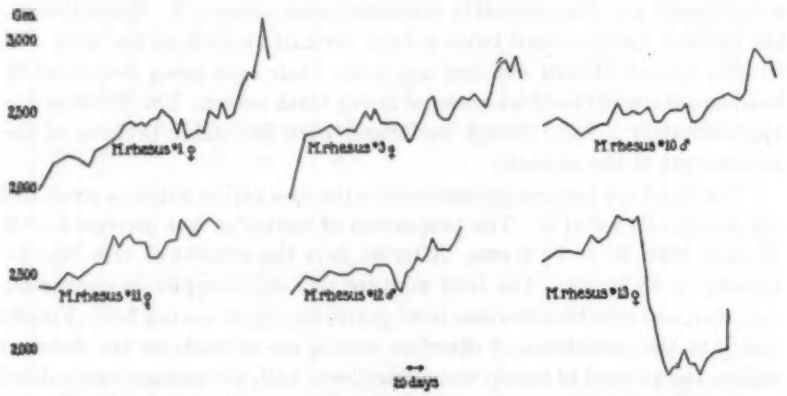

Chart 1. Growth curves of monkeys on ration containing 175 to 700 units of vitamin A.

That the six controls, with the exception of the one which was suffering from a helminth infection, gained weight and remained in excellent health, while eleven animals on an identical ration, except that the butterfat was treated by aeration and heating, invariably lost weight and died, seems to indicate that the most important factor in the outcome was the almost complete absence from the food of the latter group of the labile constituent of the butterfat, vitamin A. The histological findings in the animals support this conclusion, as will be described further on.

The six monkeys which were placed on the deficient diet at the beginning of the experiment lived for periods of 111 to 312 days, an average of 178 days, as compared with 131 days for the five animals which were started on the diet three months later. It is to be noted that the amount

of vitamin A in the ration was reduced from 12 units to 6 a month after the second group was placed under experiment, hence they received only 6 units of vitamin A during the greater part of the experimental period.

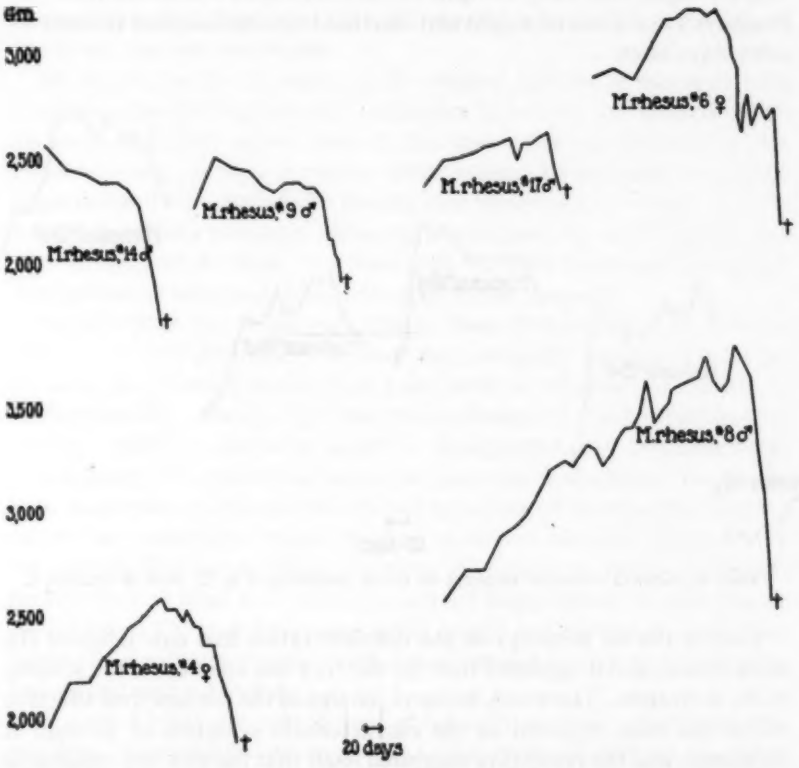

CHART 2. Growth curves of monkeys on ration containing 6 to 12 units of vitamin A.

EFFECT ON THE GENERAL HEALTH

During the first three months no differences could be detected between the animals on the low vitamin A diet and the controls. All the animals were well and active, and individual variations in rate of growth were common to both groups.

The first animal to show signs of illness was Monkey 14, which had failed to make any gain in weight, and which developed diarrhea on the 92nd day of the experiment. This condition is not rare in *rhesus* monkeys and is usually readily controlled by the administration of milk of bismuth

for a few days. The usual procedure was followed in the case of Monkey 14, but without result, the animal losing weight and strength rapidly and dying at the end of March, after 111 days. Meantime Monkey 9 had begun to lose weight, and early in April it, too, developed severe diarrhea. In Monkeys 4 and 6 loss of weight and diarrhea began to manifest themselves a few days later.

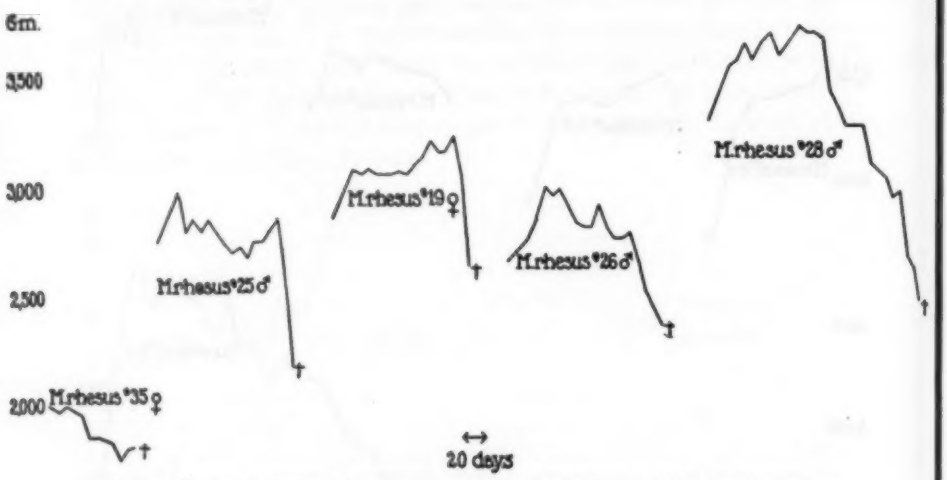

CHART 3. Growth curves of monkeys on ration containing 6 to 12 units of vitamin A.

Four of the six monkeys on the deficient ration had now followed the same course, and it appeared that the diarrhea was an effect of the absence of the A vitamin. There was, however, no sign of the conjunctival affection which has been regarded as the characteristic symptom of vitamin A deficiency, and the possibility suggested itself that the diet was unsuitable in some way; that there might be irritation of the digestive tract due to the raw swede or to the sucrose. The sucrose had been added chiefly to make the diet more pleasing to the animals in the beginning, and since they had long since become adapted to the ration the sucrose was not needed; hence it was replaced by rice. The swede was omitted from the ration for a period of 5 days, and the amount was thereafter reduced from 50 to 25 grams. The therapeutic measure taken in an effort to control the diarrhea, when the milk of bismuth failed, was to give a teaspoonful of magnesium sulphate in warm water, followed on two succeeding days by 1 cc. doses of the following mixture; 4 oz. compound chalk powder, 1 oz. bismuth subnitrate, 0.5 oz. Brown's chlorodyne, 2 oz. Tr. krameria, 3 oz. brandy,

1 oz. Tr. peppermint, 0.5 oz. Tr. capsici, 1 oz. Tr. lavender comp., water to make 1 pint.

Microscopic examinations of the stools of affected monkeys, made on several occasions, failed to reveal any diagnostic features. The only protozoa found were flagellates (Trichomonas). A bacteriological study of the stools was not attempted.

Monkey 6, the fourth animal of the original deficient group to develop diarrhea, after having lost half a kilogram in weight, was treated within 24 hours with 7 cc. of cod liver oil, the dose being repeated on the four following days. After a week the stools became formed, and the animal appeared well for a period of a month; then dysentery developed, and the animal lived only five days. Monkey 9 was given 20 cc. of cod liver oil after it had had diarrhea for a week and was very weak and emaciated. The treatment appeared to have no effect in this instance.

On the 138th day of the experiment, when Monkey 13 of the control series developed diarrhea, it seemed that we might be dealing with an epidemic of dysentery rather than a symptom of deficiency of vitamin A. In Monkey 13, however, the therapeutic treatment yielded satisfactory results. After 11 days the diarrhea disappeared, and treatment was discontinued. The condition developed again two weeks later. Treatment with magnesium sulphate was followed by a dose of the diarrhea mixture on two successive days, and 5 to 10 cc. of milk of bismuth was put daily into the distilled water which was given the animal to drink. The stools became formed after two weeks, the animal began slowly to gain weight (it had fallen 750 grams below its initial weight and more than a kilogram below the maximum weight which it had attained), and in two months had gained half a kilogram.

Monkey 13 was the only animal of the control group which ever had diarrhea. No gross lesions were found when it was killed, on the 251st day of the experiment, but microscopic examination revealed the presence of parasites in the large intestine and in the tongue epithelium. The other tissues were normal.

The eleven animals on the deficient diet, with one exception, all sooner or later developed colitis which was followed by death. The exception was Monkey 35, which died rather suddenly after having been on the diet only 74 days. The cause of death was not determined by the autopsy findings. No diarrhea had been observed.

The only striking variation in the clinical changes occurred in the case of Monkey 17, which had very marked and widespread edema for two weeks before death. This observation is of interest in view of the fact that

a similar condition was recorded as having occurred in one of the two monkeys which Harden and Zilva (33) kept until death on a diet deficient in fat-soluble vitamins.

Monkey 8 lived 134 days longer than any other monkey of the original "deficient" group. It eventually succumbed to the colitis, which was of the severest type in this instance.

Eye Conditions

After the animals had been on the diet for 91 days, and before any signs of illness had become evident, the original twelve, 6 of which had been on the diet containing 12 units of vitamin A and 6 on that containing 175 to 700 units (48), were inoculated with a saline suspension of the excised right conjunctiva of *Macacus rhesus* 72 (36), which had developed marked trachomatous lesions as a result of inoculation with cultures of *B. granulosis* 276 days previously, and in which the lesions showed no sign of regression. Three monkeys on the regular stock diet were inoculated at the same time with the same material, which was therefore distributed among 15 monkeys. The inoculations were made into the left eye and according to the technique used by Noguchi;—that is, the material was injected subconjunctivally near the border of the tarsal conjunctiva of the upper lid, and the tarsus was also scratched with the charged needle of the syringe. Earlier inoculations from the left eye of *M. rhesus* 72 had been successful, but the tissue taken for the present experiment apparently contained very few micro-organisms, since only one animal inoculated developed lesions. This animal was one of the three normal stock monkeys injected; the lesions appeared in 17 days and in 4 months became moderately severe; they still persist after 17 months. The two groups of animals on the special diets failed to respond to the inoculation.

The three surviving animals of the group on the deficient diet and the 6 controls were inoculated again 2 months later, the material in this case being a pooled saline suspension of the excised tarsi of 3 animals showing marked trachomatous lesions. Two of the three animals on the low vitamin A diet were already ill when inoculated and lived only 3 weeks. The third, which lived 5 months longer, developed no lesions, nor did any of the 6 controls.

The period of incubation in monkeys inoculated with *B. granulosis* varies from 2 weeks to 3 months (36). All but one of the 6 animals on the deficient ration was dead before the lapse of the maximum incubation period. The failure of the monkeys to develop the lesions of *B. granulosis* may have been unrelated to the condition of the monkeys. The infecting

material used in the first instance may have been taken too late in the course of the disease and for that reason contained too few organisms to produce infection, especially when distributed among many monkeys. No conclusion can be drawn regarding the relative susceptibility of the two groups of animals; the relation of diet to susceptibility to the eye disease can be determined only by repetition of the experiment with more favorable infecting material.

The absence of spontaneous eye lesions is of interest in itself, however, in view of the fact that xerophthalmia is readily produced in some animals and is rather generally regarded as having a specific relation to vitamin A starvation. It is noteworthy that Wolbach and Howe did not observe eye lesions in guinea pigs which died after two months restriction to a diet almost free from fat-soluble vitamins, and which showed the histological changes characteristic of vitamin A deficiency as demonstrated in rats.

Autopsy Findings

Complete autopsies were carried out on all the monkeys of the experiment. The tissues, except the conjunctivae, were fixed in Zenker's fluid. For the conjunctivae, Regaud's fluid was used. Sections were stained with Giemsa's solution and with hematoxylin and eosin.

The total absence of fat was striking. Examination of the tongue gland, the accessory sinuses, and the mastoids for pus proved negative in all the monkeys, though these pathological conditions were frequently found in rats fed on the same diet (38). The conjunctivae, which had shown slight congestion for a few days as a result of inoculation, but soon returned to normal, were smooth and pale when removed at autopsy.

The severity of the intestinal condition varied somewhat in individual instances. Macroscopic lesions of the intestine were rarely seen. One small area of ulceration was found in the colon of Monkey 25 and one ulcer about 5 mm. in diameter in the colon of Monkey 8. In Monkeys 14 and 9 the condition in the colon was not severe enough to have produced gross lesions, and the areas taken for section appeared normal on microscopic examination. Monkeys 26 and 35 died under circumstances such that they were not autopsied until several hours *post mortem*, hence sections of intestine were not made. In Monkeys 6, 8, 17, 25, and 28 the mucosa of the colon was redder than normal, but there was no extensive hemorrhage and no obvious ulceration.

Histological Findings

Severe ulcerative colitis (Fig. 1), the ulcers being so small as to be demonstrable usually only by microscopic examination, was present in

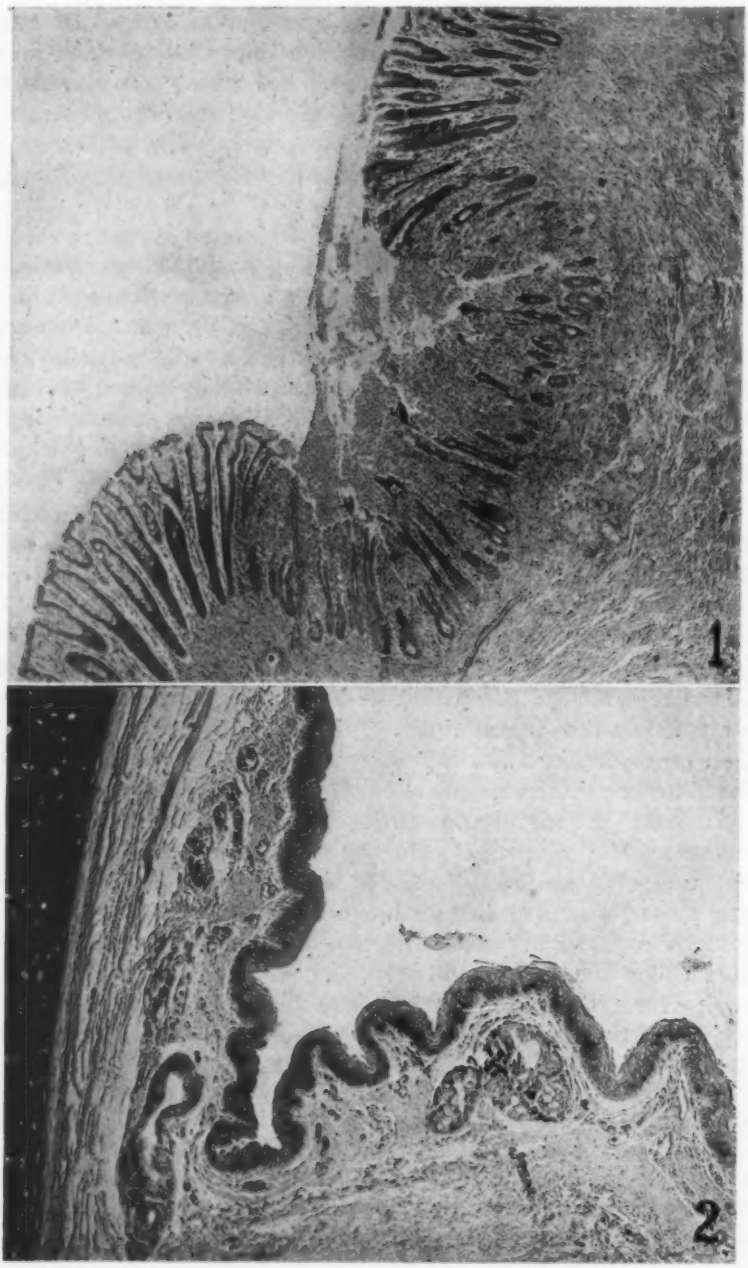

FIG. 1. Colon, Monkey 25. ×50. Showing the ulceration.

FIG. 2. Trachea, Monkey 14. ×150. Showing loss of cilia and keratinization of epithelium

Monkeys 6, 8, 17, 25, and 28, and less severe lesions of the colon were found in Monkeys 4 and 19. In Monkey 8, which survived 312 days on the diet, the only lesions found, gross or microscopic, were those of acute ulcerative colitis.

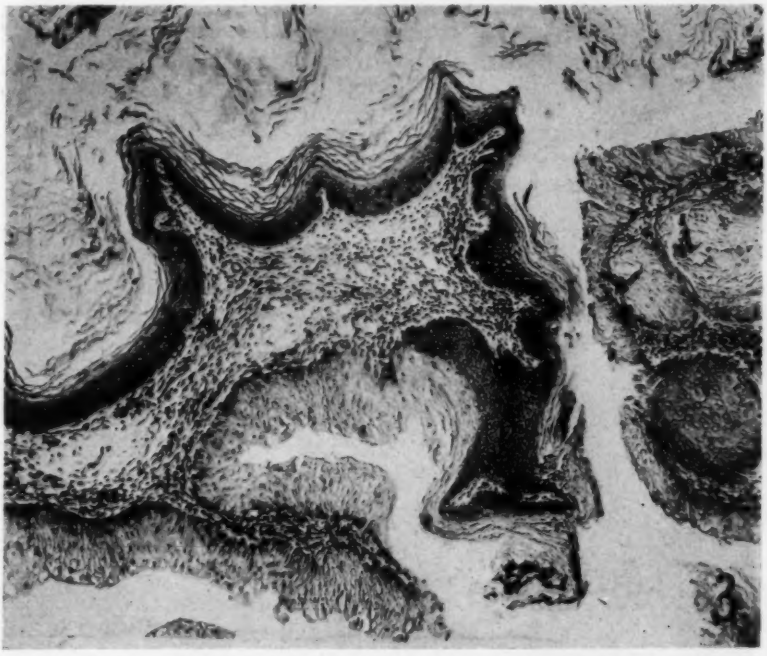

FIG. 3. Bladder, Monkey 28. ×150. Showing partial keratinization.

The epithelial metaplasia, described by Wolbach and Howe (9) and Goldblatt and Benischek (10) as characteristic of deficiency of vitamin A, was found in one or another tissue in 9 of the 11 animals on the A-deficient diet (Table II, Figs. 2, 3 and 4) and was entirely absent in the controls. The histological examinations of the preparations were made by Dr. Fred W. Stewart, of Memorial Hospital, New York City.

DISCUSSION

The data obtained in the experiment described must be presented without much attempt at interpretation. The first object of the experiment was the adjustment of the nutritional factors in such a way that the results obtained could be definitely ascribed to shortage of a single factor in

the diet. It was not practicable to make the diet entirely free from vitamin A; the amount present in the swede, however, was quantitatively determined to be very low, as had been shown by Steenbock and Gross (44).

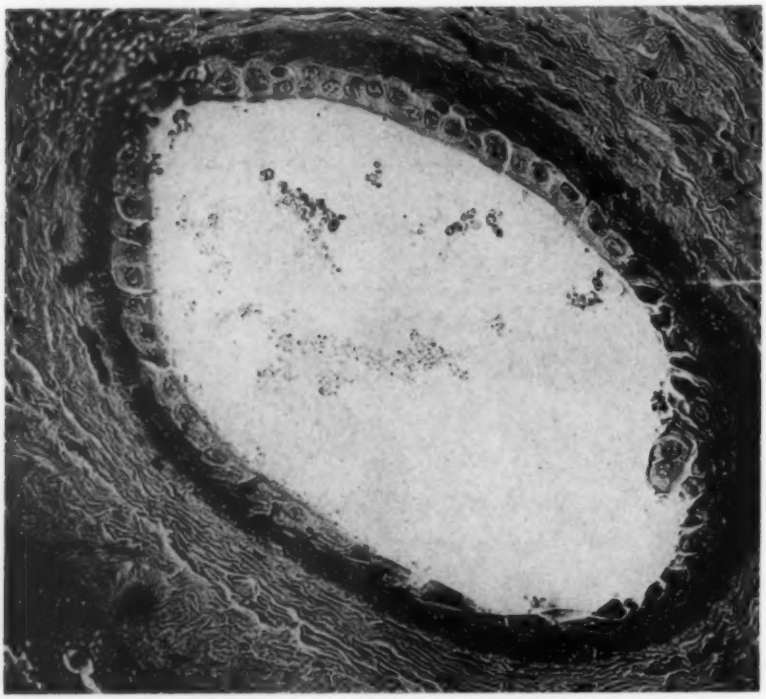

FIG. 4. Duct of salivary gland, Monkey 25. ×420. Showing replacement from below of cuboidal by squamous epithelium.

The amount of yeast given could hardly have failed to provide sufficient of vitamins B and G. No careful histological study was made to exclude rickets,* and none seemed necessary, inasmuch as the animals were receiving appreciable amounts of vitamin D in the aerated butterfat and in the swede, and were irradiated twice weekly.

It was realized at the outset that a nutritional study carried out on monkeys would be attended with difficulties, since we knew almost nothing of the nutritional history of the animals used and very little regarding the

* Dr. A. F. Hess, who was kind enough to examine the radiograms, stated that in his opinion the question of rickets was excluded.

normal rate of growth and normal life span of monkeys.* The data obtained indicate in general the rate of growth to be expected in monkeys of the species used. They also show that monkeys, like rats, may have considerable reserves of vitamin A, since these remained in good health for periods of 3 to 6 months, and in one instance for more than 9 months, on a diet containing very little of this vitamin.

With regard to the signs of depletion of vitamin A reserves in monkeys, we can say that in one respect, at least, the response of the monkey is the same as that of the rat and the guinea pig, namely, in the epithelial metaplasia which manifests itself particularly in the respiratory and genito-urinary tracts. The tissue changes were perhaps less extensive than in rats and guinea pigs.

The total absence of eye lesions in the monkeys, and of infections of the sinuses, middle ear, and tongue glands, as compared with the rather frequent occurrence of these signs in rats, is of special interest. The same observation has been made by Wolbach and Howe with respect to the guinea pig. In view of the delicacy of the monkey, as compared with the rat, however, and the possibility that the monkey succumbs to the intestinal condition before the development of other symptoms, one cannot be altogether sure that the monkey may not, under certain conditions, manifest the nutritional failure in other ways.

On the other hand, it may be that in the intestinal condition which immediately preceded death in ten of the eleven monkeys on the deficient ration, we have the characteristic effect of depletion of vitamin A in this animal. The occurrence of a non-fatal dysentery in one of the control animals confirms common experience with regard to the susceptibility of monkeys to infections of the intestinal tract. In the case of the control monkey, to be sure, a parasite was detected in sections of the intestine, while sections from the monkeys dying of colitis showed no obvious cause of the condition. Detailed bacteriological study of the stools of the monkeys during life might or might not have thrown light on the cause of the condition. It may be noted that in the colitis of "war edema" (49, 50) the bacteriological findings failed to account satisfactorily for the condition, which "rarely occurred among well-fed prisoners, though they were exposed to the infection, if this was an infectious diarrhea," and which

* We are indebted to Dr. Carl Hartmann, of the Carnegie Institution, Baltimore, for the following statement with regard to the life span of *rhesus* monkeys: "Specimens have lived in zoological gardens to my knowledge as long as 23 years . . . . . It is possible that they may live to the age of 30; the life span is therefore about a third that of man, and puberty occurs also in about a third of the human period."

TABLE II

M. rhesus No.	Sex	Period survival Days	Observations during life			Presence of keratinization of epithelium								Other pathological findings
			Loss of weight	Dysentery	Edema	Trachea	Bronchi	Esophagus	Salivary glands	Renal pelvis	Bladder	Ureter	Reproductive system	
14	♂	111	+	+		+	−	−	−	−	±	±	+	No gross changes. No microscopic changes in intestine.
9	♂	140	+	+		+	−	+	−	−	−	+	−	Abscess in lung. No changes in lung outside area of abscess.
17	♂	150	+	+	+	+	−	+	−	−	+	−	−	Colon: Ulcerative colitis. Kidney: Thickening of capillaries of glomerular tufts; casts in tubules.
6	♀	178	+	+		+	+	±	−	−	+	−	+++	Lung: Mild bronchopneumonia. Colon: Ulcerative colitis. Bone marrow: Anemia. Kidney: Casts in tubules.

TABLE II (*continued*)

4	♀	178	+	+		+	−	−	−	−	+	−	+	Colon: Acute inflammatory infiltration of lymphoid nodules.
8	♂	312	+	+		−	−	−	−	−	−	−	−	Colon: Acute ulcerative colitis.
35	♀	74	+	−		±	−	−	−	−	−	−	−	
25	♂	124	+	+		+	−	−	+	−	++++	+++	−	Colon: Acute ulcerative colitis.
19	♀	124	+	+		+	−	+	−	−	−	−	+++	Colon: Chronic toxic colitis without ulceration.
26	♂	138	+	+		+	−	−	−	−	++++	−	−	
28	♂	194	+	+		+	−	−	−	−	+++	−	−	Colon: Acute ulcerative colitis.

was often fatal. The rather striking recovery of Monkey 6 after brief treatment with cod liver oil, and the onset of the fatal colitis a month later, are suggestive.

Conclusions

Observations are reported on eleven monkeys kept until death on a ration containing only 6 to 12 units of vitamin A daily, and on 6 monkeys maintained on a similar ration, except that it contained 250 to 700 units of the vitamin *per diem*. The rations were exactly similar, except that in the case of the deficient ration the butterfat used had been aerated for 8 hours at 120°C. and contained too little vitamin A to be detected by quantitative tests in rats.

The first symptom of illness noted in the monkeys receiving the low vitamin A ration was loss of weight, which was followed sooner or later by loss of appetite and finally by colitis and death. In one animal extensive edema was present shortly before death. There was no xerophthalmia, sinusitis or mastoiditis, and tongue abscesses were not found in any of the animals.

Histological study of the tissues of the monkeys showed keratinization of epithelial tissue in one or more sites in 9 of the 11 animals. In a few instances the cornification was extreme, but in no instance were the conjunctivae involved in the keratinizing process.

Six monkeys on the control ration, with one exception, remained well and gained weight. One animal developed a non-fatal dysentery, from which it appeared to have recovered when killed. The intestine showed no gross changes at autopsy, but m croscopic examination revealed parasites in the intestinal wall. These parasites were not detected in the intestines of the animals on the deficient ration which succumbed to colitis.

Acknowledgments

We wish first to acknowledge our indebtedness to Dr. Hideyo Noguchi, in whose laboratory the foregoing experiment was carried out. Although Dr. Noguchi sailed for Africa before the work was begun, it was his interest which made its execution possible.

To Dr. C. A. Doan, of the Rockefeller Institute for Medical Research, who was kind enough to read the roentgenograms of the lungs of the monkeys, and to make a special study of the lungs in the case of Monkey 6, we wish to express our thanks.

Finally, to Dr F. W. Stewart, of Memorial Hospital, who examined the

microscopic preparations and established the diagnosis of vitamin A deficiency, we are especially grateful.

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DOES THE NICKEL DISSOLVED FROM THE CONTAINER DURING PASTEURIZATION CATALYZE THE DESTRUCTION OF THE VITAMINS OF MILK?

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DRINKER, *et al.*, (1) state that Geerkens in 1883 recovered 0.022 gram metallic nickel from two liters of milk that had stood eight days at room temperature in a nickel container. This amounts to 11 parts per million. According to Donauer (2) sweet milk at room temperature dissolves only one-tenth as much nickel as at the pasteurization temperature. On standing for eight days at room temperature milk would develop high acidity and it would seem that more than 11 parts per million should be dissolved since solutions of greater acidity dissolve nickel faster than neutral or alkaline solutions. Drinker points out that analyses of small amounts of nickel made before the development of the colorimetric method utilizing potassium di-thio oxalate are open to question. Donauer states that sweet milk at the pasteurization temperature dissolves 0.07 mg. of nickel per sq. cm. of surface exposed in 24 hours.

A most thorough review of the literature by Drinker *et al.* (1), concerning the occurrence, biologic and physiologic action, and toxicology of nickel and its various compounds, fails to shed any light upon its ability to catalyze the destruction of vitamins. The use of nickel as a catalyst in the hydrogenation of oils suggests that it might also catalyze the destruction of vitamins during the pasteurization of milk. Furthermore, the work of Hess (3) suggested, and the later work of Hess and Weinstock (4) indicated, a catalytic destruction of vitamin C in milk by copper. Since nickel is being used more extensively in industrial machinery, and especially in dairy manufacturing equipment, it seemed important to investigate the possibility of such a destruction. If only a comparatively small per cent of the vitamin content of milk or other foods were destroyed by such a catalysis, this would in the aggregate be of very great importance in human nutriton.

EXPERIMENTAL

Source and Quality of Milk. Certified milk averaging better than 4 per cent fat was obtained iced from a high grade farm near Rochester and was stored in a refrigerator at 34° F until pasteurized and, following

pasteurization, until fed. The herd from which this milk was obtained was composed of Holsteins and Guernseys in all stages of lactation and was fed on a modern dairy ration of silage, hay, and grain. In summer the cows were upon pasture supplemented by grain. The night's milk was cooled and stored in a tinned copper holding vat (inspected to see that no copper was exposed) until the morning's milk was cooled and added, resulting in a milk of remarkably unifom composition. Most of the variations common to the milk of an individual cow were eliminated by pooling the daily production of a large herd.

Pasteurization of Milk. A container of *Berndorf-rein* nickel 16 cm. high and 12 cm. in diameter and a Pyrex beaker of approximately the same dimensions were filled with milk, placed on ringstands in a hot water bath, heated by gas plates to the pasteurization temperature (145° F), and held for 30 minutes. The milk in the two containers was stirred continuously during heating by motor-driven glass rods, both at the same speed.

Because of the greater specific heat and conductivity of nickel, it was necessary to heat the milk in the Pyrex beaker to 120° F before placing the nickel container in the water bath. Both would then reach 145° F at nearly the same time. Care was taken to keep the surface free from the coagulum, which forms at the liquid-air line, to approximate the conditions in commercial pasteurization. In this way full opportunity was given for oxidation to proceed if such a reaction should occur.

Nickel Content of Milk. A sample of this milk, pasteurized in glass, when analyzed by the method of Fairhall (1) showed no nickel, while milk pasteurized in nickel showed 15 parts of nickel per million. A second pasteurization in the same container without washing caused solution of 18 parts nickel per million or 18 mg. Ni per liter of milk.

At the former rate 0.0038 mg. nickel was dissolved per sq. cm. in thirty minutes. If solution had continued at this rate for 24 hours, which is improbable, 0.18 mg. per sq. cm. would have been dissolved, or more than twice that found by Donauer. At the end of a pasteurization period of thirty minutes the walls of the container were coated with a blackened precipitate of milk solids. The fact that during the second pasteurization 20 per cent more nickel was dissolved than during the first, indicates that in commercial milk plants when a pasteurizer is used continuously for a half day or even longer without washing, the rate of solution might be considerably greater than in this experiment.

For this container 12 cm. in diameter and 16 cm. high the ratio of cubical contents to surface exposed to the milk was 0.39 sq. cm. to 1 cc., while for a continuous flow pasteurizer in the 2 in. pipes in which heating occurs

this ratio is 2 sq. cm. to 1 cc. and in the holding pipes of 5¾ in. diameter the ratio is 1.44 sq. cm. to 1 cc. For the holding type of pasteurizer of 300 gallons capacity the ratio is as low as 0.11 sq. cm. to 1 cc. Milk pasteurized by the continuous flow method would be subject to greater exposure than in the container used in this experment, while in a large vat with revolving coils, the unit of surface exposed per unit of milk would be one-third that in our container.

Since *Berndorf-rein* nickel is more soluble in organic acid than rolled, drawn, cast, or electrolytic nickel, it seemed that full opportunity was being afforded for solution and subsequent catalysis to occur during the pasteurization process if such action occurred under commercial conditions.

The nickel container before use for pasteurization purposes had a well-finished surface, perfectly smooth to the eye except for the marks left by the spinning process. After it had been used once or twice a week for pasteurization for twenty months, there were pits uniformly distributed over the surface which were plainly visible to the unaided eye, thus demonstrating the corrosive action of milk on nickel.

Vitamin A Experiments

Vitamin A Deficient Diet: For the investigation of the possible destruction of vitamin A by a catalytic action of nickel a basal diet of the following composition was used:

Commercial corn starch	76%
Casein (A Free)	18%
Agar agar	2%
Mineral salts (5)	4%

The casein was an Argentine product of very high quality purchased from Atterbury Brothers of New York. It was extracted continuously with hot alcohol for one week, dried, finely ground, and heated in an electric oven for one week at 95° to 100° C with stirring twice daily to assist in oxidation of any remaining traces of vitamin A. A feeding trial showed this to be free of the vitamin B complex according to present accepted Standards. The mixed ration was irradiated for thirty minutes with stirring at twenty inches from a quartz-mercury vapor lamp (Hanovia) to provide vitamin D.

Technique: Rats were obtained at approximately 3 weeks of age at 35 to 40 grams weight and placed in screened bottom cages to prevent access to feces. The basal A-free diet was weighed in twice weekly and the surplus weighed back at the succeeding feeding. The animals were fed 0.5

gram starch free yeast daily to supply vitamin B. They were also weighed twice weekly and weights charted on individual record cards.

Protective Method: A group of rats was obtained from the Albino Supply Company at 35 to 40 grams in weight and placed in groups of from 2 to 6 on each of the three kinds of milk (raw, pasteurized in glass, and pasteurized in nickel) on 5 cc., 10 cc., 15 cc., and 20 cc. levels of milk feeding. These rats were fed milk from the beginning of the experimental period. It was expected that even if there should be considerable destruction of vitamin A, the upper levels would still be high enough to provide for some growth. After twelve weeks of feeding there were no significant differences and therefore subsequent groups were placed on a 2 cc. level. The composite growth curves for these latter groups indicate that 2 cc. of milk do not provide sufficient vitamin A to provide for normal growth for more than 2 weeks but differences between the growth rates for the three groups were so small that they did not exceed the variation within the groups and so could not be considered significant. There were no external evidences of vitamin A deficiency other than lack of normal growth.

Curative Method. Preliminary Experiments: It was evident that if there was any destruction of vitamin A it was too small to be detected by the protective method. Accordingly a lot of rats from our own colony, weighing from 35 to 40 grams, was fed the basal A-free diet supplemented with 0.5 gm yeast daily until their body stores of vitamin A became depleted. They were kept on this ration until their weight became stationary for three weeks or fell below that of the third week previous. However, if xerophthalmia or diarrhea developed before they reached this weight, milk feeding was begun immediately. Five cubic centimeters of milk proved too high a level as recovery and growth were too rapid to reveal small differences. The rest of the animals were divided into three groups, for the three kinds of milk, and these groups in turn were subdivided and fed 1.0 cc., 1.5 cc., and 2.0 cc. respectively of milk. There were seven or eight animals in each group. The lower levels proved too low to cause rapid recovery and a steady increase in weight. The differences in weight between the animals on the 2 cc. level were neither sufficiently large nor uniform to be conclusive although there appeared to be an unfavorable effect due to nickel.

Definitive Experiments: Eighty rats averaging 33 grams in weight were purchased from the Albino Supply Company and depleted of their stores of vitamin A, as was the preliminary group.

While the preliminary group declined at six weeks at approximately 100 grams, this group did not decline until 8 weeks at 120 grams weight.

Those which declined first showed such a rapid fall in weight and such extreme symptoms that it seemed advisable to subdivide the group and begin milk feeding at once, as 2 cc. were not sufficient to cause recovery and restore the growth rate for animals of this size; consequently the groups as a whole do not show decline prior to milk feeding. The preliminary groups did not have equal average weights at the beginning of milk feeding, and this circumstance constituted an error for which no correction could be made. Sherman and Burtis (6) state that for vitamin A assay work only rats that do not differ greatly in weight should be used and advise a mini-

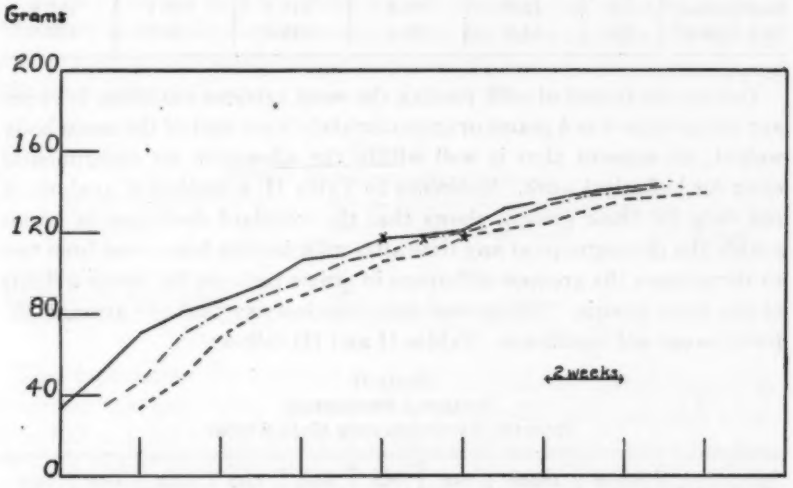

CHART 1.—Composite growth curves for rats fed raw milk, milk pasteurized in glass, and milk pasteurized in nickel for the cure of vitamin A deficiency.

Raw ——————————— Pasteurized in Nickel- - - - - - - - - - - -

Pasteurized in Glass -·-·-·-·-·-·-·- X Began milk feeding.

mum weight of 70 to 75 grams and a maximum weight of 100 grams. They found with a limited allowance of vitamin A an inverse relationship between the body weight and the rate of gain.

The definitive groups were divided as evenly as possible with respect to litters, sex, and condition so that the average weight and deviation from the mean would be comparable for the three groups. Reference to Chart 1 will show that the composite growth curves for the three groups were almost identical up to that time. During the six weeks of milk feeding the average growth rate for the three groups was so nearly the same that there is no significant difference. Table I shows the average

body weights for the three groups at significant age periods and Chart 1 the graphic record of their weights.

TABLE I
MEAN WEIGHTS OF RATS ON THE VITAMIN A EXPERIMENT

Group	No. Rats	Initial Weight	4 wks.	Beginning of milk feeding 8 wks.	3 wks. of milk feeding	6 wks. of milk feeding
		gms.	gms.	gms.	gms.	gms.
Raw	25	33.3	87.1	117.5	132.0	142.6
Past. in glass	25	32.9	89.4	118.5	133.1	142.9
Past. in nickel	25	33.0	88.5	118.9	131.6	139.9

During the period of milk feeding the most extreme variation between any two groups was 4 grams or approximately 3 per cent of the mean body weight, an amount that is well within the allowance for experimental error for biological work. Reference to Table II, a statistical analysis of the data for these groups, shows that the standard deviation in grams within the three groups at any time after milk feeding began was from two to three times the greatest difference in grams between the mean weights of the three groups. This proves quite conclusively that the average differences are not significant. Tables II and III follow:

TABLE II
VITAMIN A EXPERIMENT
STANDARD DEVIATIONS FROM MEAN WEIGHT

Kind of Milk	No. of animals	Initial Weighing	8th wk.	9th wk.	10th wk.	11th wk.	12th wk.	13th wk.	14th wk.
		gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Raw	25	3.4	11.0	11.1	10.5	11.3	11.6	11.7	12.2
Past. in glass	25	2.6	11.3	9.5	11.3	11.1	11.3	14.8	14.0
Past. in nickel	25	2.5	10.7	10.5	11.1	9.8	9.0	9.4	9.4

TABLE III
VITAMIN A EXPERIMENT
PERCENTAGE COEFFICIENTS OF VARIABILITY

Kind of Milk	No. of Animals	Initial weighing	8th wk.	9th wk.	10th wk.	11th wk.	12th wk.	13th wk.	14th wk.
Raw	25	10.2	9.3	9.4	8.6	8.5	8.5	8.3	8.5
Past. in glass	25	8.0	9.5	8.0	9.0	8.3	8.1	10.5	9.8
Past. in nickel	25	7.6	9.0	8.7	8.9	7.4	6.6	6.8	6.7

A study of the standard deviation and percentage coefficients of variability shows that the three groups were selected satisfactorily and that the data became more concordant as the feeding period progressed. The percentage coefficients of variability were smaller for the group receiving milk pasteurized in nickel than for the group receiving milk pasteurized in glass, and became increasingly more satisfactory as the end of the feeding period approached. The numbers of rats developing xerophthalmia and the average time required for recovery were also comparable for the three groups.

Table IV shows that the differences in consumption of basal diet and therefore in total food consumption, since the same amount of milk was fed each group, are negligible and indicates the comparative uniformity of the three groups.

TABLE IV
WEEKLY FOOD CONSUMPTION IN GRAMS

Groups	Raw	Past. Glass	Past. Nickel
Average of entire period	66.2	66.0	67.4
Average during milk feeding	66.3	66.6	67.8

The explanation for the fact that the preliminary groups began to decline at 100 gms. while the definitive groups did not reach this stage until 120 gms. lies in the difference of their source. Those used in the preliminary period were from our own stock while those in the latter experiment were from the Albino Supply Company and were fed a different ration, presumably higher in vitamin A content, resulting in greater vitamin A body stores. The observation of Sherman and Burtis relative to the existence of an inverse relationship between the body weight and rate of gain with a limited allowance of vitamin A was confirmed, especially by the rate of decline of the animals of the definitive group prior to milk feeding. They state further that males and females may be used interchangeably if the growth rate is approximately 3 grams per week. Since the gains were of this order and there seemed to be no consistent difference in the growth rate of the two sexes, they were averaged together to form composite curves.

The collective results of these experiments indicate no destruction of vitamin A by catalytic action during the pasteurization process unless it has been compensated by some favorable action now unknown.

Vitamin B Experiments

Vitamin B Deficient Diet: The basal diet was the same as that employed for the vitamin A experiments, with the exception that the casein was purified to remove vitamin B. This was accomplished by washing the casein continuously for a half week with 0.1 per cent acetic acid and for the remainder of the week with distilled water. The casein was then dried and finely ground. Control rats fed this diet plus daily additions of three drops of cod liver oil died in 14 days showing it to be very low in, if not entirely devoid of, vitamin B.

Technique: Rats were obtained from the Albino Supply Company at 3 weeks of age and placed in screen-bottom cages which prevented access to feces. They were fed basal diet *ad libitum* supplemented by 3 drops of cod liver oil daily in glass dishes to supply vitamins A and D.

Protective Method, Preliminary Experiments: Groups of four to six rats were placed upon 2 cc., 5cc., 8cc., and 10 cc. levels of milk feeding for each of the three kinds of milk. Those receiving milk on the 2 cc. and 5 cc. levels died too soon to manifest characteristic symptoms of polyneuritis. Those on the 8 cc. level made fairly rapid growth at first but later declined in weight and developed marked polyneuritis, while those on the 10 cc. level did not develop polyneuritis within eight weeks.

These animals received milk produced during the months of August and September which would contain less vitamin B than that produced in June when the vegetation was more luxuriant.

Definitive Experiment: A confirmatory group also secured from the Albino Supply Company at 41.9 grams was placed on the 8 cc. level the following March with 24 animals on each of the three kinds of milk. Table V gives the mean weights of the three groups at representative points throughout the feeding period and Chart 2 shows the composite growth curves for the three groups.

Table V
Mean Weights of Rats on the Vitamin B Experiment

Group	No. Rats	Initial	2 wks.	4 wks.	6 wks.	9 wks.
		gms.	gms.	gms.	gms.	gms.
Raw	24	42.3	69.5	72.0	74.9	74.0
Past. in glass	24	41.2	64.6	64.6	64.6	63.6
Past. in nickel	24	42.2	66.8	67.5	67.7	65.3

It will be noted that the group receiving raw milk made definitely greater and more uniform gain than the other two groups receiving pasteurized

milk. These two groups follow the same general course and parallel each other so closely that the differences can not be considered significant. Of the 24 animals on raw milk 13 developed polyneuritis at an average of 55.5 days of milk feeding while the remaining 11 never developed even first symptoms. Of the group receiving milk pasteurized in glass, 23 developed first symptoms at an average of 51.7 days of milk feeding while of those receiving milk pasteurized in nickel 23 developed first symptoms at an average of 52.1 days of milk feeding. The two groups developed more

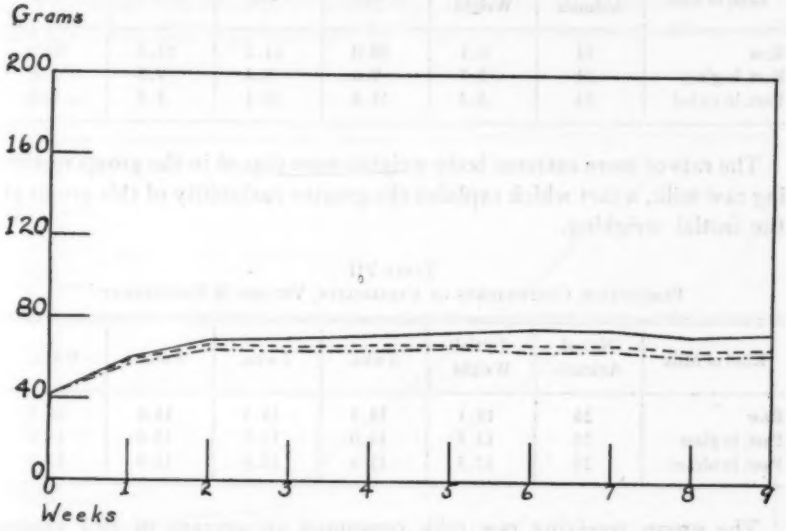

CHART 2.—Composite growth curves for rats fed milk, milk pasteurized in glass, and milk pasteurized in nickel as a source of vitamin B.
Raw ————————————
Pasteurized in Glass -·-·-·-·-·-·-·-·-·-·-·-·-
Pasteurized in Nickel - - - - - - - - - - - - -

extreme polyneuritis terminating in a spastic condition without revealing any appreciable difference. Had there been any greater destruction due to the presence of nickel it could hardly have failed to be evident. Thus it will be seen that pasteurization destroyed part of the antineuritic factor (B) but that nickel did not increase this destruction. The development of dermatitis and the shedding of hair were evident in all three groups to the same extent, showing that there was no greater destruction of the pallagra-preventing factor (G) by contact with nickel.

The following statistical analysis (Table VI) shows that the body weights of the groups were satisfactory at the beginning of milk feeding. The two groups receiving pasteurized milk were almost equally concordant from the standpoint of standard deviation and also with respect to the percentage coefficient of variability as seen in Table VII.

TABLE VI
STANDARD DEVIATION IN GRAMS, VITAMIN B EXPERIMENT

Kind of Milk	No. of Animals	Initial Weight	3 wks.	5 wks.	8 wks.	9 wks.
Raw	24	8.1	10.0	11.2	11.3	10.9
Past. in glass	24	5.7	9.0	8.4	9.3	8.8
Past. in nickel	24	5.3	11.3	10.1	7.7	8.0

The rats of more extreme body weights were placed in the group receiving raw milk, a fact which explains the greater variability of this group at the initial weighing.

TABLE VII
PERCENTAGE COEFFICIENTS OF VARIABILITY, VITAMIN B EXPERIMENT

Kind of Milk	No. of Animals	Initial Weight	3 wks.	5 wks.	8 wks.	9 wks.
Raw	24	19.1	14.3	15.3	15.8	14.7
Past. in glass	24	13.8	14.0	12.7	15.0	13.8
Past. in nickel	24	12.5	17.0	15.0	11.9	12.2

The group receiving raw milk consumed an average of 29.7 grams basal diet per week while the groups receiving milk pasteurized in glass and milk pasteurized in nickel consumed 27.7 gms. and 27.1 gms. respectively. It is well known that limiting the intake of foods containing vitamin B lowers the food consumption. The lower food consumption of the groups receiving pasteurized milk is doubtless due to thermal destruction of a part of the antineuritic factor.

A study of growth rate, standard deviation, percentage coefficients of variability, and consumption of basal diet, fails to show an increased destruction of vitamin B due to the influence of nickel.

VITAMIN C EXPERIMENTS

Vitamin C Deficient Diet: The basal diet fed to the guinea pigs was that recommended by Sherman (7) and had the following composition:

Rolled oats	29.5
Wheat bran	29.5
Sodium chloride	1
Skim milk powder	30
Butter oil	10

The skim milk powder was prepared by the spray process by the Merrell-Soule Company and was heated in an electric oven at 95° to 100° C. for four hours to destroy all vitamin C.

Technique: Guinea pigs weighing approximately 250 grams were placed on the basal diet supplemented by 10 grams cabbage and 20 cc. milk

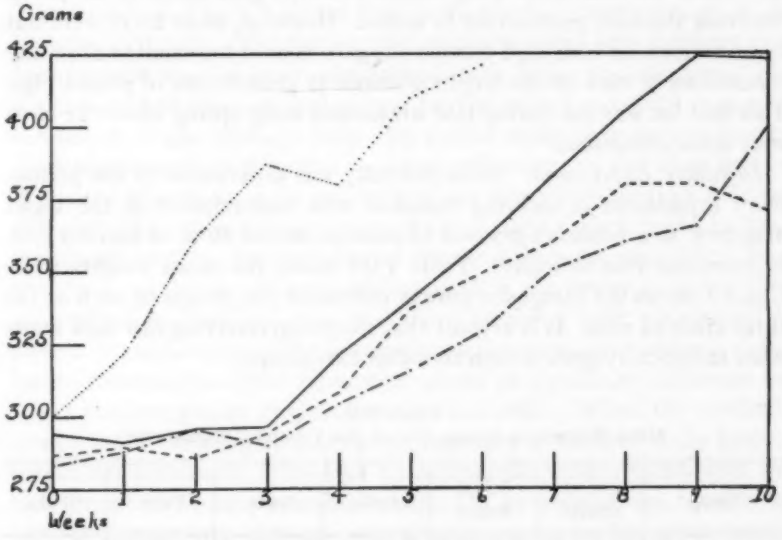

CHART 3.—Composite growth curves for guinea pigs fed raw milk, milk pasteurized in glass and milk pasteurized in nickel as a source of vitamin C. The control animal received 10 grams of cabbage daily as its source of vitamin C.

Raw ——— Normal .....
Pasteurized in Glass -·-·-·-·- Pasteurized in Nickel - - - - - -

daily. When they reached 300 grams the cabbage was withdrawn leaving the milk as the sole source of vitamin C. The basal diet was fed *ad libitum*. All animals were divided between the three groups as evenly as possible with respect to size, sex, growth rate, and source. The three groups were fed simultaneously and from the same day's milk to eliminate any errors

due to differences in weather, variations in milk or basal diet, or to variations in value of the vitamin supplements.

Weighings were made and charted biweekly.

Milk was fed in glazed porcelain dishes to avoid contact with metals.

Preliminary Experiments: Preliminary groups were started on the 20 cc., 30 cc., and 40 cc. levels of the three kinds of milk in an attempt to find a critical level for milk feeding. On the 20 cc. and 30 cc. levels there was an evident destruction of vitamin C due to pasteurization but no apparent difference between the two groups receiving pasteurized milk. On the 40 cc. level there was a much greater difference between the group receiving raw milk and those groups receiving pasteurized milk. On this level there did seem to be a significantly smaller gain made by the group receiving the milk pasteurized in nickel. However, since there were but seven animals on each kind of milk, the gain seemed too small to draw any conclusions in view of the large variations in growth rate of guinea pigs. This first lot was fed during late winter and early spring when the cows were upon winter feed.

Definitive Experiment. Some difficulty was experienced in the preliminary experiment in securing complete milk consumption at the onset of scurvy, so a definitive group of 72 animals was fed 20 cc. of summer milk to overcome this difficulty. Table VIII shows the mean weights while Chart 3 shows the composite growth curves for the groups on each of the three kinds of milk. It is evident that the group receiving raw milk made more satisfactory growth than the other two groups.

TABLE VIII
MEAN WEIGHTS OF GUINEA PIGS ON THE VITAMIN C EXPERIMENT

Group	No. of animals	Initial weight	4 wks.	6 wks.	8 wks.	10 wks.
		gms.	gms.	gms.	gms.	gms.
Raw	24	294	321	360	405	423
Past. in glass	24	283	304	329	360	399
Past. in nickel	24	287	308	349	380	371

The mean weights up to the eighth week indicate that the effect of nickel was not detrimental. The drop in weight at 10 weeks seems somewhat significant at first but a study of the individual data at this time shows it to be relatively unimportant. Of the group receiving raw milk 7 weighed more than 460 gms. while 2 weighed less than 300. Of those receiving milk pasteurized in glass 4 weighed over 460 grams and 5 under 300, while of

those receiving milk pasteurized in nickel only one weighed over 460 grams and only one under 300 grams when they died of scurvy or when they were killed at 10 weeks. The small average drop of 9 grams (2 to 2.5 per cent of body weight) is much smaller than the average variation within either of the groups receiving pasteurized milk and so becomes insignificant. Also the number of animals in each group receiving pasteurized milk was reduced by death from scurvy to 16 at the eighth week and to 6 at the tenth week. The flatness of the three curves in Chart 3 for the first three weeks is due to the withdrawal at the beginning of the experimental period of the daily allowance of 10 gm. cabbage which was the only change in the ration at that time. Apparently the withdrawal of this bulky food necessitates a readjustment of the alimentary tract.

Reference to Chart 3 shows that pasteurization destroys part of the vitamin C of milk. The flatness of the growth curve also indicates that the raw milk was fed in amounts considerably below the optimum. The positive control was a typical animal fed the same basal diet but supplemented by 10 gm. cabbage daily. Up until 5 weeks of protective feeding there was no marked difference in the growth curves of the three groups. While the growth rates for guinea pigs are more variable than for rats, still with 24 animals on each group the composite curves should be comparatively reliable. One pig died of scurvy during the experiment from the group receiving raw milk while 7 died in the group receiving milk pasteurized in glass and 5 in the group receiving milk pasteurized in nickel. Consideration of the scorbutic symptoms of those animals dying before termination of the experiment shows no significant difference between the two groups receiving pasteurized milk. When the scorbutic symptoms of the animals that came to autopsy at the end of the feeding period are considered, there was no appreciable difference between the two groups receiving pasteurized milk. On an average the fragility of bone and occurrence of rosary were slightly greater for the group receiving milk pasteurized in glass, while the other symptoms were equally manifest. Those receiving raw milk exhibited decidedly less fragility of bone while the other symptoms showed approximately the same severity as manifested by the two groups receiving pasteurized milk. This must be due to the greater weight attained and the consequently greater amount of vitamin C required to maintain the animals in normal condition. The deficiency did not exist long enough in those receiving raw milk to produce fragility of bone but did exist long enough to produce more acute symptoms. The difference in the growth curves of the two groups receiving pasteurized milk from the fifth to the ninth week was approximately

6 per cent. This difference at first might seem significant but reference to Table IX of statistical data shows the percentage deviation from the mean to exceed this difference in growth. Inspection of this table shows that the group receiving raw milk was more satisfactory than the groups receiving pasteurized milk. The maximum increase in per cent deviation for this group is equal to the per cent difference in growth rate of the two groups receiving pasteurized milk as seen in Chart 3. However, the increase in per cent deviation for the latter groups exceeds this figure and shows it to be insignificant. The per cent deviation for the group receiving milk pasteurized in nickel becomes more concordant as the experiment progresses in marked constrast to the group receiving milk pasteurized in glass. In no way can these results be interpreted as showing a detrimental effect due to nickel.

TABLE IX
VITAMIN C EXPERIMENT
PER CENT DEVIATION FROM MEAN

Kind of milk	Initial weight	2nd wk.	4th wk.	6th wk.	8th wk.	10th wk.
Raw	7.4	9.5	12.5	13.9	12.3	12.0
Past. in glass	8.4	12.4	18.8	20.5	18.0	22.6
Past. in nickel	8.6	10.2	16.3	14.0	9.9	10.1

Hotchkiss (8) found that 0.65 gm. $NiCl_2$ (0.2925 gm. metallic Ni) per liter of culture media, equivalent to about 300 parts per million, was toxic to *B. coli*, that 0.13 gm. $NiCl_2$ (0.0585 gm. Ni) limited growth, and that 13 mg. $NiCl_2$ (5.85 mg. Ni) equivalent to 5 or 6 parts per million actually stimulated *B. coli* to greater growth. This indicates that nickel under some conditions plays a beneficial role in the metabolism of protoplasm. Hart (9) and coworkers and also McHargue *et al.* (10) have shown that copper assists iron in the regeneration of hemoglobin of anemic rats fed on an exclusive milk diet. Titus, Cave, and Hughes (11) have shown that manganese aids copper and iron in this same role. The fact that nickel is used as a catalyst in the hydrogenation of oils and that it induces a tallowy or metallic flavor (7) in butter made from cream pasteurized in nickel suggests that it might play a role similar to that ascribed to copper and manganese. It seems unlikely that nickel has catalyzed the destruction of vitamins in milk unless there has been a compensation due to the presence of nickel.

Summary and Conclusions

1. The nickel content of milk pasteurized in a nickel container was 15 parts nickel per million while the same milk pasteurized in a glass vessel contained no nickel.

2. A total of 390 rats and 140 guinea pigs was utilized for these experiments.

3. In testing the catalytic destruction by nickel of each of the vitamins A, B, and C, three groups of animals were fed a ration otherwise adequate and supplemented by raw milk, milk pasteurized in glass, and milk pasteurized in nickel respectively, to supply the vitamin in question.

4. There was no appreciable destruction of vitamin A by pasteurization in either a glass or a nickel container.

5. The antineuritic factor of the vitamin B complex was partially destroyed by pasteurization but there was no evidence of a catalysis of the destruction by nickel.

6. Vitamin C was partially destroyed by pasteurization but nickel did not seem to increase the destruction.

7. Determination of standard deviation and coefficients of variability shows the data to be satisfactory and to become more concordant as the experiment progressed. The differences between the two groups receiving milk pasteurized in glass and milk pasteurized in nickel are shown not to be of greater magnitude than the deviation within the groups.

8. Unless it can be demonstrated that nickel *per se* is beneficial to animals on these vitamin deficient diets, it is impossible to interpret the data as indicating any catalytic destruction of vitamin by nickel during the pasteurization process.

A study of the above summary and a study of the rate of development of deficiency diseases, acuteness of symptoms, autopsy findings, and growth curves, do not indicate that nickel dissolved from the container during pasteurization catalyzes the destruction of vitamins A, B, or C during the pasteurization process.

I wish to express my appreciation to Drs. J. R. Murlin, and H. A. Mattill for advice and guidance in the conduct of this investigation.

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THE EFFECT OF LONG CONTINUED PARATHYROID ADMINISTRATION UPON THE BODY CALCIUM AND PHOSPHORUS OF ALBINO RATS

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IT IS a well established fact that the injection of parathyroid extract into certain animals raises the concentration of blood calcium. In some cases at least, as has been shown by Greenwald and Gross (1926), this hypercalcemia is taken care of by an increased excretion of calcium. They make the following statement: "The data presented can leave no doubt but that the administration of parathyroid extract does lead to an increased excretion of phosphorus and calcium and that this increased excretion can continue for a long period of time. The calcium must be derived from the bones. No other tissue contains sufficient calcium to have supplied the total *increased* excretion—."

If this depletion of calcium and phosphorus is substantial enough to seriously impair the body store of these elements it should be possible to demonstrate this fact by long continued parathyroid administration, an analysis of the animals so treated, and a comparison of such values with the calcium and phosphorus content of normal controls. Such was the purpose of the present investigation.

The albino rat was chosen for a preliminary investigation of this subject for a number of reasons. The animal is small enough to make an analysis of the entire body feasible, it tolerates relatively large doses of parathyroid without any disturbing effects, and it is possible to obtain quite consistent results with a well-bred strain of this animal.

EXPERIMENTAL

All of the rats used in this investigation were from one strain receiving Sherman's Diet 13 (Sherman and Campbell 1924) *ad libitum*, which consists of whole milk powder, whole wheat and salt; and, in addition, a daily feeding of fresh meat or vegetables. During the period of this experiment the animals used received Diet 13 only.

Healthy young in litters of 8 to 12 animals each were used. Certain animals from each litter were given daily subcutaneous injections of parathyroid extract, and the remainder of the litter was retained for normal controls. Care was exercised to match carefully the experimental with

the control animals as to size and sex. Growing animals were used in this work as it was felt that any tendency of the parathyroid to deplete body calcium and phosphorus would be more readily observed in growing animals than in adults. Over the experimental period the animals approximately trebled their original weights. It has been shown that during the first few months of life the calcium and phosphorus content of the albino rat increases more rapidly than the weight (Sherman and MacLeod 1925, Sherman and Quinn 1926). Thus during the period of this experiment the body calcium and phosphorus increased at least three times their original amounts.

In the first series (Group B) the animals received 0.1 ml. of a proprietary parathyroid preparation,* 15 Hanson units (Hanson 1928), daily from 28 to 60 days of age. In the second series (Group C) the animals received 0.1 ml. of the same preparation morning and evening from the time they were 21 to 60 days of age. Careful growth records were kept. At 60 days of age each animal was killed, the contents of the alimentary tract removed, weighed, and discarded. From these data the net weight of each animal was determined by subtracting the weight of the contents of the alimentary tract from the gross weight. Each animal was slowly burned in a silica dish over a low flame and finally ashed in an electric muffle furnace at a dull red heat. The ash was dissolved in dilute hydrochloric acid and made up to a definite volume, from which aliquots were taken for analysis. Calcium was determined by the McCrudden method

TABLE I

AVERAGE CALCIUM AND PHOSPHORUS CONTENT OF 60 DAY OLD MALE RATS

	No. of cases	Average net weight	Calcium in body		Phosphorus in body	
		gm.	gm.	%	gm.	%
Group A—Normal rats	11	158.9	1.325	0.834	0.904	0.569
Group B—Rats which received 0.1 ml. parathyroid extract daily from 28 to 60 days of age	3	162.8	1.364	0.838	0.929	0.571
Group C—Rats which received 0.1 ml. parathyroid extract twice daily from 21 to 60 days of age	4	145.3	1.207	0.831	0.822	0.566
Groups B and C combined	7	152.8	1.274	0.834	0.868	0.568

* Paroidin, Parke Davis & Co.

(1911), and phosphorus by the method described in the Methods of Analysis of the Association of Official Agricultural Chemists.

The weight of body calcium and phosphorus and the per cent of each of these elements in each animal was calculated. The averages for all animals are given in Tables I and II, the males being shown in Table I and the females in Table II.

TABLE II

AVERAGE CALCIUM AND PHOSPHORUS CONTENT OF 60 DAY OLD FEMALE RATS

	No. of cases	Average net weight	Calcium in body		Phosphorus in body	
		gm.	gm.	%	gm.	%
Group A—Normal rats	13	122.4	1.138	0.930	0.756	0.618
Group B—Rats which received 0.1 ml. parathyroid extract daily from 28 to 60 days of age	2	109.6	1.009	0.921	0.666	0.608
Group C—Rats which received 0.1 ml. parathyroid extract twice daily from 21 to 60 days of age	4	121.9	1.168	0.958	0.756	0.620
Groups B and C combined	6	117.8	1.113	0.945	0.726	0.616

DISCUSSION

From an examination of Tables I and II it may be seen that the percentages of body calcium and phosphorus in the rats receiving parathyroid were not measurably different from the percentages of these elements in untreated rats of the same age, sex, and litter. The animals receiving parathyroid were normal not only in regard to percentage of calcium and phosphorus, but also with respect to growth rate, activity, and appearance. The number of cases is probably not sufficient to make a statistical examination of the data possible, but the agreement is so marked that it was not felt needful to use a larger number of animals.

The manufacturer kindly supplied a fresh, tested batch of their parathyroid extract which was used for a part of the animals. There was no difference observed between the action of this material and that of the same trade name purchased on the market.

Greenwald and Gross (1926) and more recently Tweedy and Chandler (1929) have shown that the normal rat is quite resistant to parathyroid extract. Greenwald and Gross state: "It may be that their (rabbits', rats') mechanisms for the excretion of calcium function more readily and they, therefore, never develop a dangerous hypercalcemia."

The dosage of parathyroid administered in this work is the clinical dose recommended for children. Although the dose used was relatively large, it may be that it was not massive enough to cause an increased excretion of calcium and phosphorus. Or it may be that the diet, being fairly rich in both calcium and phosphorus, furnished enough of these elements to permit normal deposits of them in the tissues in spite of an increased excretion.

Summary

The long continued administration of parathyroid extract to normal growing albino rats receiving a normal diet had no measurable effect upon the percentages of body calcium and phosphorus in the animals.

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A MULTIPLE-CHAMBER RESPIRATION APPARATUS FOR RATS AND OTHER SMALL ANIMALS

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THE literature describing the various types of respiration apparatus which have been used for rats has recently been reviewed.[1] The method usually employed in rat studies is that of Haldane. The time required for weighing and connecting the various members of the absorbing train in this apparatus is such that ordinarily but one fairly long experimental period can be obtained in a day. An apparatus with which two or three short, consecutive periods can be secured enables a better control of the accuracy of the measurements. The rat apparatus used by Benedict and MacLeod[1] permits of periods one or two hours in duration,

[1] Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, 343. To the literature cited by Benedict and MacLeod should also be added the following references, which contain descriptions of apparatus for rats and small animals:

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H. Kunstmann (see book by M. B. Schmidt, "Der Einfluss eisenarmer und eisenreicher Nahrung auf Blut und Körper," Jena, 1928, p. 83), of Schmidt's institute in Würzburg, has described an apparatus much on the same principle as that of Wesson (*Jour. Biol. Chem.*, 1927, LXXIII, 499) but adapted for mice. He states that the apparatus has been in use for a long time. E. B. Hanan (*Science*, 1929, N. S., LXX, 582) has recently described an automatic microspirometer of ingenious construction, which he states is being used by J. O. Ralls of the University of Buffalo in studying the metabolism of rats. No details of the construction of the chamber or results with rats have thus far been published. At the time of going to press our attention is called to the researches of Males and Magaz (*Boll. d. Soc. Ital. di Biolog. Sperim.*, April 17, 1929, IV, fasc. 4) in the laboratory of Baglioni at Rome. These investigators studied the metabolism of the albino rat at various environmental temperatures, by means of an apparatus constructed on the Regnault-Reiset principle.

and experiments made with it have generally comprised three consecutive periods of such length. Both of these types of apparatus, however, demand the attention of an assistant during most of the period of observation, and with but one experiment a day progress is slow. One reason for the slow accumulation of data with these two types of apparatus has been that the measurement of the carbon-dioxide production was considered essential. From innumerable observations on rats 24 hours after food was removed, it is now known that the basal respiratory quotient of this animal is close to 0.72. If an average respiratory quotient of 0.72 is assumed for an animal in the post-absorptive state, the error in computing the heat production from the oxygen consumption is insignificant, because the caloric value of a liter of oxygen varies only within narrow limits with respiratory quotients between 0.70 and 1.00. With humans the present-day tendency in respiration experiments is to disregard the carbon-dioxide measurement and base all calculations of the metabolism upon the oxygen consumption, which can be measured in short periods. Consequently, in the multiple-chamber apparatus here to be described (an important feature of which is facility of manipulation), the carbon-dioxide measurement, though easily made, is usually foregone and the oxygen consumption only is determined in periods of about one hour in length.

General Principle of the Multiple-Chamber Respiration Apparatus

The multiple-chamber apparatus[3] (see Figure 1) consists of four individual units, mounted compactly on the same table. Each unit consists of a hermetically sealed respiration chamber, ventilated by a small rotary blower. The carbon dioxide is removed from the air current by a suitable absorbent. A small spirometer acts as an expansion chamber and as a reservoir for the supply of oxygen. A pump of known volume is used to introduce oxygen into the closed system to replace that consumed by the animal. From the known volume of the oxygen introduced, and from the recorded time which has elapsed during the consumption of this known volume, one can calculate the apparent volume of oxygen used by the animal in a given time. Reduction of this measurement to standard conditions of 0° C., dry, and 760 mm. and multiplication of the reduced volume by the caloric value of oxygen for an assumed respiratory quotient of 0.72 gives the heat production of the experimental animal for a given period of time. Slight changes in the absorber system (see page 166) make it possible to measure also the carbon-dioxide production, when desired.

[3] This apparatus can be secured from W. E. Collins, 555 Huntington Avenue, Boston, Mass.

The individual units are so mounted that one person can easily operate all four at the same time and thus secure simultaneous measurements on four different animals.

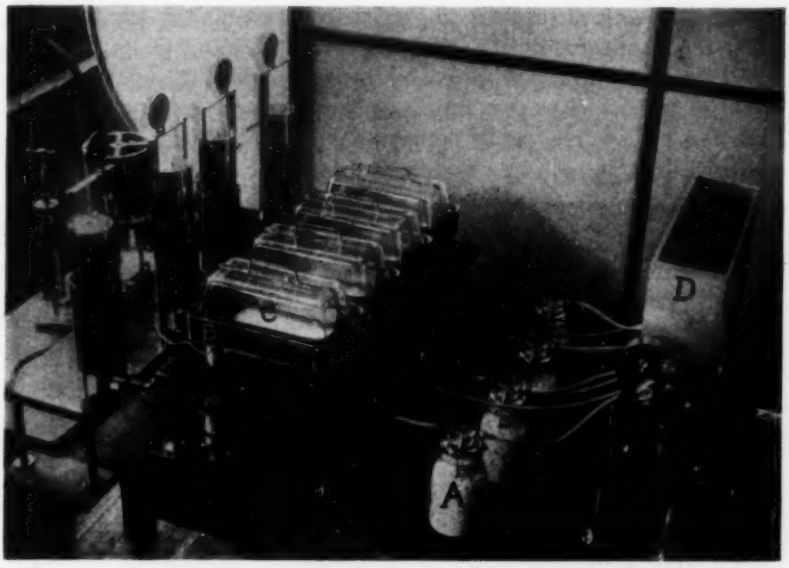

FIG. 1. Multiple-chamber respiration apparatus for small animals. Each unit consists of a respiration chamber with water-sealed base, B, and glass cover, C, connected with a spirometer, S, a soda-lime container, A, and a small rotary blower immersed in oil in the blower box, D. The pump, P, measures the amount of oxygen introduced into the spirometer. The kymograph, K, is part of the activity-recording device.

DESCRIPTION OF AN INDIVIDUAL UNIT OF THE MULTIPLE-CHAMBER SYSTEM

Respiration chamber. The chamber has a metallic base (B, Figure 1), provided with a water seal. Its outside dimensions are 29.2 by 16.5 by 5.5 cm. and the inside dimensions are 24.0 by 11.0 by 5.4 cm. for the length, width, and height, respectively. The connections for the air current and the activity recording apparatus pass through the bottom of this base. The upper part or cover, C, of the chamber consists of a standard baking-dish (for bread) of pyrex glass. This, when inverted, fits into supporting U-hooks in the water seal, covers the cage in which the rat is placed, and forms a well-lighted respiration chamber with visible interior. The thick pyrex glass is a good insulator. The space in the interior of the chamber

may be enlarged by raising the glass cover a maximum of 45 mm. This is done by supporting the cover upon shallow U-hooks or loops in the water seal.

Animal cage. The rat is placed in a cage (not shown in Figure 1) of perforated metal, rectangular in shape except that at the rear a section of the top slopes slightly towards the bottom. The cage is large enough to accommodate a full-grown rat, but is also provided with partitions by means of which the smaller rats may be shut into a space comfortably large but not so large as to permit of undue movement. Soldered to either side of the cage at two different places are two sets of vertical grooves into which partitions of perforated metal may be slid. These grooves are so placed that the space inside the cage may be adjusted for either a small or a medium sized rat. To prevent the rats from eating their own feces, especially when fasting, the cage has a false floor (6 mm. above the real floor) or wire screening with 1/4-inch (6 mm.) mesh. Through this the feces fall. The sloping section of the top at the rear of the cage forms a door, which is hinged to the rest of the top and is fastened to the side of the cage, when closed, with a hook or clip.

Spirometer. To provide an expansion chamber for the closed-circuit respiration apparatus a spirometer, S, with a light-weight, well counterpoised bell is used. The bell is suspended over an aluminium pulley with fine fish line, and a counterpoise with pointer passes over a vertically supported, steel millimeter scale. Each millimeter length of the cylindrical bells thus far made for this apparatus corresponds to a volume of 4.58 cc. Ordinarily the spirometer is used only as a null instrument, the experimental periods beginning and ending with the bell at the same low level. There is therefore a minimum amount of air in the bell, and hence a thermometer for the spirometer is not necessary.

Ventilating system. A respiration chamber of this size is usually ventilated at such a slow rate (from one to two liters per minute) that a blower of slow speed is required. Because of the slow shaft revolution called for in this apparatus, a special flat spring is necessary to press the blades inside the blower out against the walls of the compression chamber. For general use the blower must produce the maximum ventilation which, for rats, is ordinarily about two liters per minute. With a standard maximum ventilation, the effective ventilation may be reduced at will by attaching a by-pass to the two sides of the blower. The directly connected spirometer offers an excellent method for measuring the rate of ventilation. Thus, with the motor rotating at the usual speed, with the by-pass opened to the desired degree, and before the glass cover of the chamber

is inserted in the water seal of the base, the operator places his finger over the opening of the discharge from the blower to the base of the chamber. Air then passes from the blower directly to the spirometer. Since the volume of the spirometer bell per millimeter of its length is exactly known, if the time required to fill the spirometer is also known, the rate of ventilation is easily determined.

Carbon-dioxide absorbent. As is customary in the use of all respiration apparatus at the Nutrition Laboratory, soda-lime is employed to remove the carbon dioxide from the air current. This reagent absorbs carbon dioxide with great avidity and high efficiency. Ordinarily we prefer the dry type of soda-lime, the preparation of which has already been described.[3] This soda-lime is much drier than the patented soda-lime frequently used in measurements of the respiratory exchange. It has the advantage, therefore, of removing not only the carbon dioxide from the air current but also a large part of the water vapor, thus keeping the humidity of the air in the chamber fairly low. If drier air is desired, a bottle containing calcium chloride can be inserted in the air line. The soda-lime container is a quart, glass, preserve jar, A, fitted with a metal cover with closing device modified to meet the special conditions of the small animals. (See Figure 1.) In the general use of the respiration apparatus, that is, when the oxygen consumption alone is being determined, this bottle is not weighed, for the soda-lime absorbs water as well as carbon dioxide from the air current.

Measurement of the oxygen consumption. The Nutrition Laboratory has developed a simple type of piston pump[4] (P, Figure 1) which can be relied upon to discharge into the ventilating system an exact volume of oxygen with each full stroke. The diameter of the cylinder and the length of the piston stroke being known, a simple calculation gives the apparent volume of gas discharged per stroke, about 370 cc. From this apparent volume is calculated the volume of oxygen, reduced to 0° C., dry, and 760 mm. This calculation is based upon the temperature of the pump barrel (recorded at the time when the oxygen is introduced into the closed system) and the prevailing barometric pressure, with due consideration of the fact that the oxygen inside of the pump is saturated with water vapor at the temperature of the pump. Indeed, care is always taken to

[3] Atwater, W. O., and F. G. Benedict, *Carnegie Inst. Wash. Pub.*, No. 42, 1905, 29; Benedict, F. G., *Abderhalden's Handb. d. biolog. Arbeitsmethoden*, 1924, Abt. IV, Teil 10, 449.

[4] Benedict, F. G., *Boston Med. and Surg. Jour.*, 1927, CXCVII, 1161; *ibid.*, *Chinese Jour. Physiol.*, 1928, Report Series No. 1, 39; *ibid.*, *Abderhalden's Handb. d. biolog. Arbeitsmethoden*, 1929, Abt. IV, Teil 13, 1.

fill the pump with oxygen from a rubber bag or container, to the inside walls of which water is adhering. Thus the gas is always saturated. One complete pumpful of oxygen is introduced into the apparatus at one time.[5] Before the introduction of the oxygen, the level of the spirometer bell is noted, the experiment beginning preferably when the bell is nearly all the way down. The spirometer bell rises when the oxygen is introduced and the experiment continues until, by reason of the oxygen consumption of the animal, the bell has again assumed its original level. The time required for the bell to return to its original level is the time taken by the animal to absorb one complete pumpful of saturated oxygen at the known conditions of temperature and pressure.

Measurement of the carbon-dioxide production. Although the apparatus was designed primarily to measure only the oxygen consumption of small animals, it can be used likewise for determining the carbon-dioxide production. It can be confidently assumed that the respiratory quotient of a rat which has been 24 hours without food is close to 0.72. Hence a direct measurement of the carbon-dioxide production is usually of but little importance. We have determined the respiratory quotients of rats on many occasions with this type of apparatus. We believe that the respiratory quotient itself and especially the changes in the respiratory quotient during the transitional period immediately after the withdrawal of food can be studied much better with the exact Carpenter gas-analysis apparatus.[6] But since it is conceivable that quantitative determinations of the carbon-dioxide production may have value in certain studies, the following method for use with this multiple-chamber apparatus has been devised. The air must enter and likewise leave the carbon-dioxide absorbing train absolutely dry. For this purpose receptacles containing a drying reagent must be placed before and after the soda-lime container, the second receptacle being weighed with the soda-lime container. By means of a pair of 2-way cocks and suitable fittings inserted between the chamber and the blower, it is possible to direct the air current passing from the chamber through previously weighed vessels or U-tubes containing soda-lime and calcium chloride, or pumice stone and sulphuric acid. At the end of a given period the 2-way valves can be turned and the air current deflected at will from one set of reagent containers to a second set.

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[5] The pump rod may be marked at the midway point so that precisely one-half a stroke may be used at a time.

[6] Carpenter, T. M., *Jour. Metab. Research*, 1923, IV, 1; Carpenter, T. M., and E. L. Fox, *Jour. Biol. Chem.*, 1926, LXX, 115; Carpenter, T. M., E. L. Fox, and A. F. Sereque, *Jour. Biol. Chem.*, 1929, LXXXIII, 211.

Removal of water vapor in the air current. To avoid condensation of moisture on the inside of the glass cover, it has been found advantageous in most instances to dry the air with calcium chloride. Moisture from the water seal can be avoided by using oil. This we have not done. We have, however, minimized the influence of water vapor by placing a tray of fused calcium chloride on top of the animal's cage, inside the glass cover. To hold this tray in place, a rim has been soldered around the top of the cage. The use of this tray may at times require the elevation of the glass cover with shallow U-hooks, to secure greater clearance between the cover and the animal's cage. Drying of the air in the system has been found especially necessary when large, almost giant rats have been studied. An increased ventilation rate and the insertion of a calcium-chloride can in the circuit also helps.

Activity record. The animal's cage rests on a movable floor consisting of a small, rectangular plate of sheet brass or copper with the sides turned up 2 or 3 mm. At one end of this floor on the under side are soldered two pins, made preferably of a hard, non-rusting metal or alloy. These pins rest upon two sockets or cups of the same metal, soldered to the base of the respiration chamber. At the other end of the floor is soldered a hook. This end of the floor is suspended by a rubber band which passes under the hook and over a support on the inner wall of the base. The slightest change in the center of gravity of the animal results in a change in tension on the rubber band and causes a slight change in the elevation of the movable floor. Since the floor rests on a small rubber bulb, any slight changes in level of the floor are transmitted outside the chamber through a capillary tube and a length of rubber tubing[7] to a tambour with pointer, writing directly upon the smoked paper of a kymograph drum, K. The Collins "Chronokymograph", modified at our request to make one revolution in two hours, has been found satisfactory. When the activity is to be recorded of small animals whose weight is slight in proportion to the mass of the suspended system, it is desirable that the recording device should be extremely sensitive. For this reason we have used a light-weight straw of small caliber with a Bayliss writing point.

Accessories. To absorb any urine excreted by the animal during the period of measurement, a piece of thick, white blotting paper is placed beneath the cage on top of the movable floor. A thermometer to indicate the temperature inside the respiration chamber may be fastened by ad-

[7] The rubber tubing which we have found most satisfactory is a pure gum tubing with 1/16-inch hole and a 1/32-inch wall, sold by the Davol Rubber Co., Providence, R. I., as tubing No. 2500.

hesive tape to the top of the glass cover, on the inside, or it may be attached to the top of the animal's cage by a small metal clip with little studs on the back, which drop into the holes in the perforated metal of the cage.

The Multiple-Chamber System

The details of the apparatus, as outlined above, represent the latest modifications of the single respiration chamber previously described.[8] The important feature of the present form of apparatus is the multiple-chamber, *i.e.*, a system in which four respiration chambers, each with its own spirometer, carbon-dioxide absorbent, and rotary blower, are to a certain extent run independently. By this system it is possible to study at the same time four different animals, one in each of the chambers, and thus increase fourfold the number of experiments possible during a day, when controlled by one observer. The principle involved is exactly the same in all four units, *i.e.*, a closed-circuit respiration chamber ventilated at a suitable rate, the removal of carbon dioxide from the air current, and the automatic introduction of oxygen from a spirometer bell directly connected with the chamber.

Heating of the chambers. In the single apparatus special electric heating was necessary to maintain the respiration chamber at a temperature suitable for the experiments. In this multiple-chamber system the close proximity of the several chambers makes heating at the same time simple and more complex. The two inner chambers receive not a little heat from the two outer chambers, but the latter are much more affected by any changes in room temperature. Heating of all four chambers is accomplished by a system of electric resistances, one under each chamber. We have found satisfactory the simple 5-ampere heating unit with lamp base, such as is ordinarily screwed into portable electric heaters. Invariably external resistance is necessary to cut down and control the heating effect. Special asbestos partitions are used to confine the heat to the individual chamber or specifically to that portion of the base of the chamber lying directly beneath the cage. Since the cover of each chamber is of thick glass, of itself a good heat insulator, there is no difficulty in controlling the temperature of any of the four chambers in cold weather. Indeed, it is unnecessary to cover the chamber with a heat-insulating material to maintain the temperature at, for example, 28° C., even in a cold room. A more difficult problem has been to hold the temperature at 28° C. during warm weather. The best results have been obtained thus far by

[8] Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, 343.

placing a piece of filter paper or loosely woven cloth, such as cheese cloth or muslin, previously moistened, over the cover of the chamber. An electric hair dryer, the speed of which has been cut down with external resistance, directs a current of cold air over the chamber and advantage is thus taken of the cooling by the vaporization of water. Occasionally water is dropped upon the fabric to keep it moist.

Installation of rotary blowers. The installation of four rotary blowers, one for each chamber, was accomplished as follows. All four blowers have been placed in a cast-iron box and are driven by a common rotating shaft, with suitable gears. The box is 13 by 42 by 24 cm. in width, length, and depth, respectively. It is thus deep enough so that the blowers can be covered with sufficient oil, moderately heavy, to prevent leakage of air into the system, and any leakage of air out of the system can be detected by the air bubbles which will pass up through the oil. The main rotating shaft runs the entire length of the box, passes out through the wall at one end, and is connected by gears with an electric motor on the outside. At the point where it passes through the wall of the box, the shaft is fitted on the outside with a stuffing nut and gland, with suitable packing. This prevents leakage of oil out of the box. Along the shaft at various points are attached four small driving gears. These gears are adjusted to slide lengthwise on the shaft and are fixed with a set screw bearing on a flattened portion of the shaft so that they can be adjusted to engage with utmost nicety with the larger gears on each of the four blowers. The ratio of the small to the larger gears is 3:1. The pipes to and from the blowers are directed downward and pass out of the bottom of the box through holes, each of which is fitted with a gland, stuffing nut, and packing. Under these conditions leakage of oil from the box around the pipes is prevented. It is important that the several blowers be installed in the box so that they can be removed easily for repair or for substitution of another blower. After drawing off the oil, one can take out any blower by releasing the small driving gear on the shaft, sliding it along the shaft a few millimeters, and unscrewing at the bottom of the box the packing nuts connecting the two air pipes of the blower under consideration. When these are released, the blower can be lifted out immediately. The blowers rarely have to be changed during use. Their rate of rotation is slow, they are well lubricated continually, and should give no trouble for years at a time.

Direction of rotation of blowers. In the mounting of the blowers reduction of speed is obtained at two points. Outside the blower box reduction is made by a small gear on the armature shaft of the motor. Inside the box the gears on the common shaft are much smaller than those on each

of the separate blower shafts (ratio 1:3). We have found it advantageous to arrange the whole system so that suction is the predominant condition in each blower, that is, to have the connections such that, if a leak exists, oil will be *sucked into* the blowers rather than air forced out. Under these conditions, therefore, if a leak is present, oil may be sucked into the blower and thrown out, if in excess, on the discharge side.

Oil trap. To prevent an excess of oil from entering the rubber tubes and exterior connections, each blower is provided with a small oil trap on its exit side. This trap consists of a brass tube 5½ cm. long and 22 mm. in diameter, with a side tube near its top. The air leaving the blower is discharged into this trap, near the bottom, and passes out by the side tube. At the bottom of the trap is a spring key cock. This cock may be opened at convenient times to note whether oil is being carried over, and particularly to draw off any excess oil. A great excess of oil in the trap means that there is such a leakage into the blower that the blower should be repaired. Obviously, with this method of installation the blower may be used until two other criteria of inefficiency appear, *i.e.*, until the excess of oil carried over is such as to fill the trap and spurt over into the rubber tubes during the time allotted to one experiment or until the blowers fail to produce the desired ventilation rate. Under ordinary conditions, however, we have found that the blower with specially fitted vanes requires little attention.

Electric motor. To drive this system of main shaft and four blowers we have found a one-sixth horse power motor satisfactory. The high shaft speed (1720 r.p.m.) of the motor must be reduced, and a fibre, bakelite, or laminated gear, which lessens the noise perceptibly, has been used. A simple brass-strap guard is attached over the gears to prevent accidents. The ratio between the gear on the armature shaft and the gear on the main driving shaft is 3:1. The best rate of speed of the main driving shaft has been found to be 570 r.p.m.

Main divisions of the multiple-chamber apparatus. For purposes of handling, installation, and repair it has been found practical to mount the four different divisions of the apparatus on four separate bases. Thus, the motor-blower unit, consisting of the blower box containing the blowers and the directly connected motor with reduction gear, is substantially mounted on a heavy, wooden base. The absorbing vessels (usually consisting solely of one soda-lime bottle for each unit or, in special cases, of other types of containers such as U-tubes holding soda-lime and calcium chloride or pumice stone and sulphuric acid) rest either on a special platform or more commonly on a laboratory table. The four respiration cham-

bers proper, with their heating elements beneath, are mounted together on a strong wooden base which can be adjusted to any desired position on the laboratory table, independent of the other divisions of the apparatus. All four spirometers are likewise well mounted on a board. This board also carries a 3-way cock, through which oxygen is distributed to any one of the four spirometers, as desired. In all our use of the apparatus we have relied upon a metallic, ground-joint union for connecting each spirometer with the base of each chamber, but smooth nipples with good rubber tubing are equally useful and probably in the long run preferable. The arrangement of the four divisions of this multiple-chamber apparatus is so flexible as to allow considerable movement of the different parts, when desired. Furthermore, each section is on its own base and each is replaceable.

Advantages of the multiple-chamber system. The chief advantage of this multiple-chamber type of apparatus is that all four chambers can be employed at the same time. In addition, one or more of the chambers can be used independently without the slightest difficulty. It is also possible to alter the rate of ventilation by means of a simple by-pass around the blower. Thus the maximum ventilation rate, which is determined by the speed of the blower shaft (assuming that there is no great resistance in the air circuit), may be reduced to almost nothing by opening the by-pass. Finally, although the width and length of the water seal in the base of the chamber restricts the apparatus to animals which can be contained in a chamber of this same length and width, a metallic, rectangular box (with a glass window in the top to make the interior visible) of any desired height may be used, so long as its base fits into the water seal. Indeed, we have constructed a metallic chamber of such size as to permit the introduction of a bird as large as a common pigeon, at the same time retaining the sensitivity of the activity recording device and using the water seal of the original chamber base for the closure of this much larger chamber. The multiple-chamber system therefore lends itself to the study of the metabolism of animals ranging in size from an albino rat weighing some 60 grams to a giant rat weighing 600 grams and, indeed, to very large sizes of pigeons.

Control Tests of the Multiple-Chamber Apparatus

From the general principle of the apparatus, its construction, and the connection of its various parts, one could predict perfect functioning. But an apparatus as complicated as this may not be used uncritically without careful tests to insure the solidity and accuracy of its construction

and its capacity to measure quantitatively the carbon dioxide evolved or more particularly the oxygen absorbed by the experimental animal. A number of tests, the importance of which cannot be over-emphasized, have therefore been devised to control the perfect functioning of the apparatus.

General test for tightness. The principle upon which this apparatus is operated demands a system which is absolutely air-tight. There is no pressure inside the chamber, because it is directly connected with a delicately counterpoised spirometer bell, which serves as an expansion chamber, and the nature of the absorbent for carbon dioxide is such that no appreciable suction or pressure is developed. Nevertheless a defective connection or even a pinhole leak anywhere in the entire circuit of the ventilation system will vitiate the measurements. Hence the first test of the apparatus is a general test for tightness. Fortunately this test is simple. The glass cover is set in the water seal of the base, the soda-lime container is connected, the spirometer bell is half filled with air, and a weight of 50 grams is placed on the spirometer bell, the blower remaining motionless. A sizeable leak will be apparent instantly in the fall of the spirometer bell and of course its source must be traced. By pinching off the connections between the chamber and the blower and between the chamber and the soda-lime bottle, the chamber alone and the spirometer may be tested. If a leak is still found, the spirometer itself may be tested by closing the open end leading to the chamber and placing the weight as usual on the bell. The spirometer, if tight, may be used for testing the blower and connections and, indeed, the soda-lime bottle, independent of the chamber. If the spirometer alone is first tested, and then the spirometer connected in succession with the chamber, the soda-lime bottle, and finally the blower, one can rapidly locate any leak.

Overnight test. To make sure that the apparatus is functioning perfectly at the beginning of each day's observations, an overnight test is an imperative accompaniment of all investigations with this apparatus. At the end of an experiment, when the animal is removed from the respiration chamber, the glass cover is put back in place, the spirometer is two-thirds filled with air, and the 50-gram weight is placed on top of the bell. The position of the bell is then noted. The weight is left on the spirometer bell overnight and the next morning the position of the bell is again recorded. These two readings will rarely be identical, for aside from any change in the position of the bell which might be caused as the result of a leak, there may always be changes in the volume of the air confined in the system (including the spirometer, chamber, soda-lime container, blower, and connections) due

to changes in environmental temperature and atmospheric pressure. It has never been our experience, however, that the combined fall in temperature and rise in barometer overnight have been sufficient to account for the spirometer bell being at its lowest level. Consequently it may be concluded that *if the spirometer bell is way down in the morning after an overnight test, this is positive proof of a leak, but if the bell is not way down, no significant leak is present.* This test should be made overnight on each of the four respiration chambers.

Tests for tightness while running the motor. It is conceivable that a leak might occur when the apparatus was actually functioning, that is, when the blower was running. Hence it is customary, especially at the time of installation of the apparatus, to repeat the above tests with the blower running. A variant of these tests is to hang a weight of 50 grams on the counterpoise of the spirometer and thus control the tightness of the apparatus under conditions of slightly *decreased* pressure as well as under conditions of slightly increased pressure.

Given a tight apparatus the oxygen measurement must be correct, with the single possible error due to the incomplete absorption of carbon dioxide. Since the amount of carbon dioxide to be measured during an experiment with a small animal such as the rat is not great, incomplete absorption of carbon dioxide may be said never to occur unless a wholly inefficient soda-lime is used.

Physiological test. The use of a fasting animal to control the accuracy of a respiration apparatus is perhaps not so general as it should be.[9] In studying the metabolism of the albino rat, Benedict and MacLeod[10] found that the respiratory quotient 16 hours after food was almost invariably 0.72 or 0.73. Although the multiple-chamber apparatus was not primarily designed to measure the respiratory quotient, it can be arranged to determine the carbon-dioxide production of an animal by weight and the oxygen consumption by volume, either by means of the spirometer or the piston pump. (See page 165.) A physiological control of the apparatus can therefore be made by measuring in any one or all of the respiration chambers the respiratory quotient of an adult rat from 18 to 24 hours after the withdrawal of food. If the apparatus is operating satisfactorily, the quotient should be close to 0.73.

Alcohol check test. All good respiration apparatus or calorimeters are today accurately controlled by developing inside of the apparatus a

[9] J. R. Murlin (*Amer. Jour. Diseases of Children*, 1915, IX, 43) has suggested using a diabetic dog for this purpose.

[10] Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, 352 and 360.

known amount of carbon dioxide, heat, and water vapor, with the resulting absorption of a definite and known amount of oxygen. In many cases the measurement of the oxygen consumption alone is used as evidence of accuracy. In other cases both the carbon dioxide and the oxygen measurement serve as criteria, and obviously in calorimeters all four factors come into play. This type of test is applied to all of the well-known, closed-circuit respiration apparatus for humans and has even been employed in the control of apparatus for large animals. *The ideal conditions for such a test are the development in the respiration chamber of that amount of carbon dioxide and the absorption of that amount of oxygen corresponding to the amounts normally developed and absorbed in regular experiments with the apparatus, whether for humans or animals.* It is experimentally unsound to test an apparatus which is to be used for measuring an extremely small carbon-dioxide production or oxygen consumption by developing and absorbing several times the amounts normally to be expected. In the control of this multiple-chamber apparatus the question arose as to whether it would be possible to burn in the chamber an amount of alcohol small enough to correspond to the carbon-dioxide production (heat production) of a single adult rat. The adult rat at 28°C produces ab out 3.5 cc. of carbon dioxide per minute per 200 grams. Can a flame be arranged to burn alcohol so as to produce carbon dioxide at this low rate? Our usual arrangement in burning alcohol consists of a burner made of a pyrex glass, capillary tube with a wisp of asbestos as a wick. This burner is connected through the wall of the chamber with a carefully calibrated, 2 cc. burette, graduated in hundredths of a cubic centimeter. This burette is raised slowly either by a slowly rotating kymograph spindle or by a mechanico-chemical device.[11] This particular procedure failed utterly when we attempted to apply it to the small chamber for rats. We could not make the flame small enough. After much experimenting it was found that even when the capillary tube was drawn out to a fine point, the glass conducted sufficient heat away to make the flame easily extinguishable. Only after applying an old but simple expedient first published, we believe, by Thomsen[12] in 1882, were we successful in securing a small flame. This expedient consists in winding about the capillary tube a coil of platinum wire with a thin layer of asbestos between the wire and the tube. When this coil is heated electrically, the alcohol in the capillary is vaporized not by its own heat but by the electric heat. Thus a moderately

[11] Benedict, F. G., *Boston Med. and Surg. Jour.*, 1925, CXCIII, 583.
[12] Thomsen, J., *Thermochemische Untersuchungen*, Leipzig, 1882, IV, 16.

steady supply of alcohol vapor escapes from the capillary and burns reasonably well. With every precaution to avoid the slightest draughts inside the chamber we were able to make a few successful experiments in which we not only secured a quantitative measurement of the oxygen absorption but succeeded in keeping the flame burning the requisite time without going out, *i.e.*, burning long enough to permit of the collection of sufficient carbon dioxide and the absorption of sufficient oxygen to make the measurements reliable. In this procedure, which may properly be looked upon as a "*tour de force*," we could not reduce our alcohol flame to a point low enough to represent the carbon-dioxide production of an adult rat. Indeed, the alcohol flame produced carbon dioxide at a rate about 15 per cent greater than would a rat. But we believe that the test was sufficiently close to justify our efforts.[13]

Reliability of the Multiple-Chamber Apparatus

Two multiple-chamber apparatus of the type here described have been in constant use since December, 1927, one in a cooperative research on the albino rat with Professor Lafayette B. Mendel at Yale University, New Haven, Conn., and the other in a cooperative study of common pigeons and ring doves with Dr. Oscar Riddle at the Department of Genetics, Cold Spring Harbor, Long Island, N. Y.[14] During these two years eight rotary blowers have been in almost daily use and but one has had to be changed. No essential alteration has been made in any of the other parts of the system, and the apparatus is seemingly so rugged in construction that little, if any, difficulty is to be expected from defective connections or imperfections.

Summary

A multiple-chamber, closed-circuit respiration apparatus is described, with which one person can measure simultaneously the gaseous metabolism of four different animals in periods as short as one hour or, if desired, can study only one or two animals at a time. The apparatus is comprised of

[13] The difficulty of making an alcohol check in a small respiration chamber for rats has likewise been emphasized by Wesson (*Jour. Biol. Chem.*, 1927, LXXIII, 504). Although Wesson evidently attempted the combustion of small amounts of alcohol, the amounts actually burned are not given, and whether they approximated the carbon-dioxide production of a rat or greatly exceeded it we do not know.

[14] Reproductions of the multiple-chamber apparatus, with in some instances slight modifications, have been made by W. E. Collins (555 Huntington Avenue, Boston) for the use of Dr. M. O. Lee of the Harvard Medical School, Dr. H. M. Evans of the University of California, Dr. N. F. Blau of Cornell University Medical School, and Dr. G. E. Collip of McGill University. A second apparatus has also been installed at Cold Spring Harbor for the use of Dr. Oscar Riddle.

four individual units. Each unit consists of a respiration chamber, a ventilating rotary blower, a spirometer serving as an expansion chamber and also as a reservoir for the supply of oxygen, and a container for soda-lime, in which the carbon dioxide is absorbed from the ventilating air current. Oxygen is introduced into the spirometer from a pump of known volume. From the recorded time which has elapsed during the absorption of this known volume of oxygen by the animal, and from the records of pump and chamber temperatures and atmospheric pressure, can be calculated the volume of oxygen consumed at 0°C. and 760 mm. per hour. Slight changes in the absorber system make it possible to measure also the carbon-dioxide production and hence the respiratory quotient.

Each chamber is provided with a device for the graphic registration of activity. The water vapor in the air of the closed system is removed by fused calcium chloride placed in a tray on top of the animal's cage inside the chamber. The four individual units are compactly mounted on a table, with separate bases for the spirometers, the respiration chambers, and the blowers, so that readjustment of the different parts may be readily made, when desired. The four blowers are immersed in oil in a metal box and are driven by a common shaft, with suitable gears, actuated by an electric motor outside the box. An electric resistance under the metal base of each chamber and asbestos partitions between the chambers aid in controlling the temperature inside the chambers.

The accuracy of the apparatus has been demonstrated by an alcohol check test, in which a small flame was successfully kept burning to produce carbon dioxide at a rate only 15 per cent greater than the rate of carbon-dioxide production by a single, adult, albino rat. General tests for tightness of the apparatus are also described.

This multiple-chamber system lends itself to a study of animals ranging in size from a rat of 60 grams to a giant rat of 600 grams and to the larger sizes of pigeons. Two apparatus of this type have been in constant use since 1927 in cooperative investigations on rats and pigeons.

THE METABOLISM OF THE ALBINO RAT DURING PROLONGED FASTING AT TWO DIFFERENT ENVIRONMENTAL TEMPERATURES

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THE metabolic changes in a living organism are most simply studied when an animal is in a fasting condition, that is, when the absorption of previously digested food has ceased and the animal is drawing upon its own body substance. With certain animals, notably ruminants, food residues may remain in the alimentary tract for many days and, either by direct digestive activity or by bacterial or fermentative action, may be drawn upon in energy transformations for an extended time. Wild animals, especially those which migrate, ordinarily experience great changes in their food supply, and without doubt many undergo long periods with little or no food. Several of the larger animals, including man,[1] have been known to fast for thirty days or more, and some of the cold-blooded animals, especially snakes, have fasted for inconceivably long periods. Indeed, there is the well-authenticated instance of a rock python at the New York Zoological Park which refused food for twenty-one months. The smaller laboratory animals, on the other hand, are generally believed to withstand fasting poorly and hence studies of the metabolism of small animals while fasting have usually co ered only a short period.

The investigations of Goto,[2] of Terroine and Trautmann[3] and of Benedict and MacLeod[4] indicate that the basal metabolism of the rat is increased approximately 5 per cent for each degree (Centigrade) fall in environmental temperature. The extent to which the body resources of these small animals may be drawn upon during fasting will therefore be in large part determined by the environmental temperature. In most studies with rats little attention has been given to the environmental temperature, since temperature was not the special factor under-investi-

[1] Benedict, F. G., *Carnegie Inst. Wash. Pub.*, No. 203, 1915.

[2] Goto, K., *Biochem. Zeitschr.*, 1923, CXXXV, 115. Goto used the apparatus of Aszódi (*Biochem. Zeitschr.*, 1921, CXIII, 70) in the laboratory of Professor Hári at Budapest.

[3] Terroine, E. F., and S. Trautmann, *Ann. de Physiol.*, 1927, III, especially Table III, p. 436.

[4] Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, 370 and 376.

gation. Observations made on a colony of rats at Columbia University, however, have brought out the influence upon metabolism not only of the environmental temperature during the experiment itself, but likewise of the temperature at which the animal ordinarily lived prior to the experiment.[4] As a result of these observations and in order to determine how long a small animal can withstand fasting, we planned to study the metabolism of the albino rat during fasting prolonged until death and at two different environmental temperatures.

The Respiratory Quotient of the Fasting Rat

In the report of observations on rats at Columbia University[5] the longest fasting periods recorded were 64 hours. The results obtained in these short fasts imply that the glycogen storage of the fasting rat is depleted rapidly and that the respiratory quotient soon falls to a level characteristic of fat combustion. Although the investigation to be reported in this present paper did not deal with the respiratory quotient *per se* of the fasting rat, it was necessary in computing the heat production from the measured oxygen consumption to assume a fasting respiratory quotient. A study was therefore made almost simultaneously with the prolonged fasts to determine at what time interval after the withdrawal of food the respiratory quotient reaches a level indicative of the combustion of fat or body substance. The results of this special study are given, before proceeding to a consideration of the metabolism measurements secured during the prolonged fasts.

A simple respiration apparatus of small volume was immersed in a water bath in order that the environmental temperature might be controlled. Samples of the outcoming air could be collected and analyzed according to Carpenter,[6] thus permitting a rapid study of the transitions in the respiratory quotient following the withdrawal of food and the determination of the time elapsing before the appearance of a quotient representing essentially the combustion of fat. This simple respiration apparatus, which has been used both for pigeons and for rats, was demonstrated at the Nutrition Laboratory in Boston during the meeting of the XIIIth International Congress of Physiologists, August 19–23, 1929. A brief description, with diagrammatic sketch, of the apparatus has already been published.[7]

[4] Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, 343.

[5] Carpenter, T. M., *Jour. Metab. Research*, 1923, IV, 1; Carpenter, T. M., and E. L. Fox, *Jour. Biol. Chem.*, 1926, LXX, 115; Carpenter, T. M., E. L. Fox, and A. F. Sereque, *Jour. Biol. Chem.*, 1929, LXXXIII, 211.

[7] Benedict, F. G., *Bulletin Soc. Sci. d'Hygiène Alimen.*, 1929, XVII, 327.

In experiments with pigeons Riddle and Benedict[8] have found it desirable, owing to the storage of food in the bird's crop, to have the pigeons begin the fasting periods with a known amount of food in the crop, approximately 5 grams for ring doves and 10 grams for common pigeons. With these birds a fat quotient almost invariably appeared after twenty-four hours of fasting. At times, however, a relatively high quotient, in some instances approximating 1.00, was noted even as late as eight or ten hours after the withdrawal of food. At the twelfth hour of fasting the quotients were often about 0.90 but in no instance were they above 0.74 after 24 hours of fasting and usually this value appeared at the eighteenth hour.

With rats,[9] experiments were made on six different days, in which the respiratory quotients were determined immediately after the withdrawal of food to the fifth hour, at intervals of one or two hours, and also between the sixteenth and the twenty-third hour after food. The results are given in Table I.

In the two experiments on October 27 food had been available to the rats up to the time they were put into the respiration chamber, but we do not know exactly when they had last eaten. In both of these experiments the quotients were over 0.80 at the start and fell during the few hours over which the observations extended. In all other cases the quotient was below 0.80 at the start of the experiments, which usually began at least sixteen or eighteen hours after food was taken away. Twenty hours after food the quotient was in no instance above 0.75 and was usually below this point. We may conclude from our data that the respiratory quotient of the rat fasting for twenty-four hours would be in the neighborhood of 0.72 or 0.73. In view of the severe drafts made upon body material during a fast, and particularly in view of the rapid loss in weight during the first day or two, it is conceivable that a fast prolonged to forty-eight hours may actually be a detrimental feature in many researches. In other words, in the second twenty-four hours of fasting, although a large part of the glycogen has disappeared, there may be such a heavy draft upon body material as to defeat certain experimental ends. We believe, however, that the respiratory quotient of the rat after twenty-four hours of fasting is so near 0.72, on the average, that one may with confidence measure the carbon-dioxide production or the oxygen consumption only, assume a respiratory quotient of 0.72, and compute the total metabolism with a minimum error.

[8] Benedict, F. G., and O. Riddle, *This Journal*, 1929, I, 502 and 506.

[9] These rats were loaned to us through the kindness of Professor W. E. Castle at the Bussey Institution of Harvard University.

TABLE I

RESPIRATORY QUOTIENTS OF RATS DURING THE FIRST TWENTY-FOUR HOURS WITHOUT FOOD

Date	Body weight	Hours without food	Respiratory quotient
1928	gm.		
Oct. 27	155	0	0.82
		1½	.80
Oct. 27	245	0	.87
		1½	.89
		2½	.87
		4	.83
		5	.86
Oct. 29	200	16	.70
Oct. 30	185	17	.72
Oct. 30	165	17	.70
Oct. 29	260	16	.74
		17½	.70
		22	.73
Oct. 24	215	18	.72
		20	.69
Oct. 24	240	19	.78
		20	.74
Oct. 25	165	19	.73
		20	.75
Oct. 25	175	19	.74
		20	.73
		21	.74
Oct. 23	160	21	.72
Oct. 23	240	21	.72
		23	.73

Observations on rats in Hári's laboratory show that on the first and second days of fasting the respiratory quotient may be expected to be within the limits of 0.71 and 0.75.[10]

Our finding on the fasting respiratory quotient is at variance with the results reported by Wesson in 1927.[11] The ingenious apparatus used by Wesson seemingly leaves no possibility for criticism. We have been es-

[10] Goto, K., *Biochem. Zeitschr.*, 1923, CXXXV, 112.

[11] Wesson, L. G., *Jour. Biol. Chem.*, 1927, LXXIII, 504–506.

pecially interested in the excellent attempts of Wesson to secure alcohol checks with his apparatus, realizing their importance and also the difficulties of making such tests. In the small series of alcohol checks reported by him the four successive respiratory quotients obtained range from 0.72 to 0.64, and both the theoretical and the calculated averages are 0.67. In a series of respiratory quotients obtained by him on normal rats during fasting, only the values of 0.74 for rats 11 and 12 and 0.71 for rat 4 (average values) agree with the results secured at the Nutrition Laboratory. In observations with rats 9 and 10 after 72 hours of fasting, the first two quotients are low but, singularly enough, the succeeding quotients increase considerably and the highest quotient (0.80) occurs at the end of four hours. It is not our intent to criticize too sharply these results of Wesson, but our experience in studying the respiratory quotients of humans leads us to believe that Wesson's series of quotients illustrates the danger of using the respiratory quotient as an index of the character of the metabolism. If two series of quotients with fasting rats obtained in two different laboratories can vary as widely as do ours and Wesson's, the difficulty of comparing the fasting quotient with that following a superimposed factor becomes even more apparent.

Wu and Chen,[12] in studying the basal metabolism of omnivorous and vegetarian rats, report values for the respiratory quotient which more closely approximate our results, although occasionally some of their quotients are high. For a group of normal male rats they find that the respiratory quotient averaged 0.73 and for a group of normal female rats 0.74. They used a closed-circuit apparatus enabling the determination of both the carbon-dioxide production and the oxygen consumption. The experiments were made at an environmental temperature of 28° C. and the animals had been without food for from 16 to 28 hours. The results are based upon selected periods of 15 or 20 minutes' duration, in which there was no movement on the part of the animal.

Our picture with fasting rats is confirmed to a certain extent by the experience of Benedict and Riddle with fasting birds of essentially the same body weight, that is, the respiratory quotient reaches a level indicative of essentially a fat metabolism within twenty-four hours after food, and does not thereafter rise to a higher level. This picture has been shown sharply and consistently in all their work with both ring doves and common pigeons.[13]

[12] Wu, H., and T. T. Chen, *Chinese Jour. Physiol.*, 1929, III, 307 and 315.
[13] Benedict, F. G., and O. Riddle, *This Journal*, 1929, I, 506.

The rapid and complete withdrawal of glycogen from fasting rats is also shown by the admirable studies of Cori and Cori,[14] who found, both by analyses of the tissues and from determinations of the respiratory quotient, a low glycogen content in rats which had fasted for 48 hours. Their studies on the respiratory quotient indicate that after 48 hours of fasting the quotients average 0.711. The rats were kept for 24 hours before the measurement of the respiratory quotient and during the metabolism experiment itself at a temperature between 27° and 28° C., at which the minimum metabolism of these animals prevails. Even under these most favorable conditions for lessening the drafts upon body substance, a low carbohydrate storage was found after 48 hours of fasting and the respiratory quotients indicated practically no combustion of carbohydrate.

Plan of Research

This cooperative research on the metabolism of the albino rat during prolonged fasting was undertaken with the several objects in view of determining the length of life until the occurrence of death, the total loss in body weight, and the changes in the total metabolism as the fast progressed. In addition, the influence upon the body-weight losses and the length of life, during fasting, of a rapid metabolic rate produced by a low environmental temperature was studied by having one group of rats fast at an environmental temperature of 26° C. and another group at 16° C. A multiple-chamber respiration apparatus (described in a preceding article in this journal[15]) was installed at Yale University and with it metabolism measurements on the two groups of fasting rats were secured.

Animals Used

Virgin, female, albino rats from the Osborne and Mendel colony were used. Prior to the fasts these animals were fed a stock diet, which consisted of a special "dog biscuit" supplemented by fresh cabbage twice each week. In so far as possible they had lived a normal existence. The fasts occurred in the winter months, during February and March of 1928 and January of 1929. The rats measured in the respiration chamber at 28° C. (Rats I to IV) were kept for 24 hours preceding the first metabolism measurement and during the intervals between the measurements in a glass cage,[16] the temperature of which was maintained constant at not far from 26° C. The group of rats (Rats V to VIII) studied at the lower

[14] Cori, C. F., and G. T. Cori, *Jour. Biol. Chem.*, 1926, LXX, 557.
[15] Benedict, F. G., *This Journal*, 1930, III, 161.
[16] Described by Benedict, F. G., and O. Riddle, *This Journal*, 1929, I, 493.

temperature were kept in a cool room for 24 hours preceding the first metabolism measurement and between the periods of measurement at a temperature of essentially 16° C. Water was accessible to both groups of animals during the fasting period, except when they were inside the respiration chamber.

Prior to the experiments these two groups of rats were subjected to slightly different conditions. Thus, the rats that were to fast at 26° C. were brought from a nearby laboratory eleven days before the fast began, so that they might become acclimated to the conditions in the metabolism laboratory. During these eleven days each rat gained in weight on the average 15 grams. The average weight at the beginning of the fast was 222 grams. The rats that were to fast at 16° C. were brought to the metabolism laboratory only three days beforehand, and during these three days lost on the average 4 grams each. The average weight of these rats was 216 grams at the start. The rats which fasted at 16° C. therefore had an initial body weight somewhat smaller than that of the rats fasting at 26° C. The rats in both groups were essentially of the same age. (See Tables II and III.)

Discussion of Results

Length of life of fasting rats. The popular notion that small animals have a low resistance to fasting is well exemplified by the statement of Morgulis[17] that "Large animals endure, therefore, a fast much longer than smaller ones". In a list giving the average length of survival of representatives from different animal groups, headed by man at 40 days, rats are said to live two or three days. Inconsistent with this information is the reference, eight pages later in the book, to the interesting work of Böhtlingk[18] of St. Petersburg with mice fasting 6½ days.

Although several investigators have studied the metabolism of fasting rats, there is relatively little evidence as to how long the rat can actually live under continued inanition. Mitchell and Carman[19] give metabolism data for a rat that fasted for 53 hours and Wesson[20] for rats fasting 72 and 96 hours, but they do not state when the animals died. Chanutin and Silvette,[21] in studying the creatine content of tissues and blood in rats,

[17] Morgulis, S., Fasting and undernutrition, New York, 1923, 93 and 101.

[18] Böhtlingk, R. R. de., *Arch. sc. biol.*, St. Petersburg., 1897, V, 395. Böhtlingk points out that his mice fasted without water. They had the advantage, however, of fasting at a warm temperature (summer) and perhaps might have lived longer than 6½ days, but for purposes of the investigation they were killed before death occurred.

[19] Mitchell, H. H., and G. G. Carman, *Amer. Jour. Physiol.*, 1926, LXXVI, 390.

[20] Wesson, L. G., *Jour. Biol. Chem.*, 1927, LXXIII, 505.

[21] Chanutin, A., and H. Silvette, *Jour. Biol. Chem.*, 1928, LXXX, 589.

report fasts lasting as long as 11 days and state that as a rule the animals were not killed until they were very weak and on the point of death.

In the experiments of Giaja and Males,[22] who studied what they term the "*métabolisme de sommet*," fasting rats are reported to have lived 6 and 9 days. The animals were subjected to extreme cold, even being plunged into ice water, with a resultant increase in the metabolism of from 250 to 300 per cent above basal. Although these periods of extreme cold were of necessity fairly short, the rats had to struggle against this tremendous heat loss for some time after the cooling treatment had stopped. There must have been, therefore, a great draft upon previously stored material. The basal metabolism of these rats was first determined and and then, presumably on the same day, the "*métabolisme de sommet.*" Two rats withstood this treatment for 6 days and a third rat for 9 days.

Osborne and Mendel,[23] in studying the use of cotton seed as food, report experiments made on 12 control rats (six young and six adult), deprived of all food except water. The environmental temperature is not given. The adult rats survived the fast on the average 7 days and the young rats 4 days. The maximum length of life was 12 days with a male rat weighing 287 grams at the start.

Barlow and Whitehead[24] studied 21 male rats which fasted from 2 to 12 days, with water available. These animals were not allowed to live until death occurred naturally, but were killed in groups of four or five at intervals of from 2 to 12 days, in order to study the course of the changes in the blood and the body weight. Certain of the rats used by these investigators were, however, allowed to die from starvation. Professor Barlow has kindly written to us the details of these fasts, with permission to publish the information. His "original protocols yield the following observations obtained from at least three animals in each group. Under conditions of constant temperature (averaging 24° C.) with access to water, young rats (50 to 90 grams) usually die from four to six days after the beginning of the fast. Medium sized rats (125 to 150 grams) usually die after from six to nine days. Adult rats (175 to 225 grams or larger) live ten or more days, usually from eleven to thirteen days after feeding has been discontinued. Two large rats (larger than 300 grams) lived for 14½ days and another large rat for 15 days."

The duration of the fast until the occurrence of death is reported for our rats in Tables II and III (pages 187 and 188). The rats fasting at

[22] Giaja, J., and B. Males, *Compt. Rend. Soc. Biol.*, 1926, XCIV, 226.
[23] Osborne, T. B., and L. B. Mendel, *Jour. Biol. Chem.*, 1917, XXIX, 298.
[24] Barlow, O. W., and R. W. Whitehead, *Amer. Jour. Physiol.*, 1929, LXXXIX, 548.

26° C. lived longer, except in one instance, than those fasting at 16° C. Thus, three of the four rats studied at the higher temperature lived from 15 days and 20 hours to 25 days. The fourth rat lived a shorter time, dying on the ninth day. With the four rats kept at 16° C. the length of the fast varied from a minimum of 6 days and 20 hours to a maximum of nearly 13 days. Two of the rats died on the tenth day. The animals at the warm temperature therefore withstood the fast longer than those at the low temperature, with the single exception of Rat IV, which died after only 9 days. The general picture is that death took place much sooner at the cool temperature than at the warm temperature. The shorter life of the rats that fasted at 16° C. cannot be explained as resulting from undue exposure to cold. The temperature of the room in which they were kept reached a minimum of 12° C. on one occasion (although no rat died at this temperature), but for the most part averaged 16° or 17° C.

Loss in body weight of rats fasting at two different temperature levels. One of the commonest indices of the completeness of fasting, at least with humans, is the course of the loss in body weight from day to day, for it has been found that this daily loss persists with almost mathematical regularity. Relatively little has been published regarding the loss in body weight of fasting rats.

Osborne and Mendel[25] found that rats (average weight 215 grams) that died after 7 days of fasting lost on the average 39 per cent of their initial body weight and that rats (average weight 74 grams) that died after 4 days of fasting lost 35 per cent. Cori[26] found that rats fasting 48 hours lost on the average 12.9 per cent of their weight but that obviously there were variations in this loss due to the individual variability of the rats, the irregularity in food intake prior to the fast, and the uncertainty with which the initial body weights of rats can be determined.[27] Giaja and Males,[28] in studying the "*métabolisme de sommet*" of fasting rats, report the initial weights and the weights at death, from which we may compute the total percentage losses. Thus, their rat No. 1 weighed before the fast 261 grams and at death on the sixth day 174 grams; rat No. 2 weighed

[25] Osborne, T. B., and L. B. Mendel, *Jour. Biol. Chem.*, 1917, XXIX, 298.

[26] Cori, C. F., *Jour. Biol. Chem.*, 1925, LXVI, 696.

[27] It occurs to us that activity might also play a considerable role in the body weight losses during fasting. It is commonly observed that rats are often much more restless and active when on a diet deficient in any important factor than when on a complete diet. The activity of our rats which fasted at 26° C, however, did not confirm this general picture. Indeed, an examination of the activity records (Tables IV and V) for these rats shows that they became more quiet as the fast progressed, at least while they were inside the respiration chamber.

[28] Giaja, J., and B. Males, *Compt. Rend. Soc. Biol.*, 1926, XCIV, 226.

159 grams at the beginning and at death on the sixth day 119 grams; rat No. 3 weighed 251 grams at the start and 156 grams on the tenth day, when it died. The rate of loss was 33 and 25 per cent in the two 6-day fasts and 38 per cent in the 9-day fast.

Chanutin and Silvette[29] found with rats which fasted after having received 5 and 10 per cent creatine diets that the percentage loss in weight over a given time was dependent upon the initial weight and that practically all their rats died of fasting after 30 to 40 per cent of the body weight had been lost. Barlow and Whitehead[30] state that "the median weight changes (of fasting rats) are roughly proportional to the length of the period of inanition." In personal correspondence Professor Barlow also states that it has been his experience that adult rats may die at any time after they have lost 30 per cent of their body weight.

With humans, who live in surroundings which provide artificially for comfort, the environmental temperature during fasting has not been markedly changed over that to which man is accustomed. With laboratory animals the temperature of the environment is known to have a profound influence, and for this reason our studies included the effect of fasting at 26° C., which is near the critical temperature for the rat, and likewise at a distinctly lower environmental temperature of 16° C. The amount of body material katabolized during fasting is roughly proportional to the body weight loss. One could *a priori*, therefore, assume that the katabolism to sustain the fasting organism would be proportional to the loss in body weight, although one must always bear in mind the possibility of the replacement of fat by water. Since the rate of metabolism is considerably increased at the lower temperatures, a comparison of the losses in the body weights of our rats at the two different temperature levels is of significance.[31]

The body weights of our rats from day to day of the fasts, including the weights at death, are recorded in Tables II and III. Of the animals fasting at the higher temperature Rat I lost a total of 50 per cent of the initial body weight, Rat II lost 48 per cent, and Rat III lost 54 per cent. The greater loss of Rat III is without doubt due to the fact that this animal survived the fast for a longer period than did Rats I and II. If, however, we compare the weight of Rat III after 15 days and 16 hours of fasting

[29] Chanutin, A., and H. Silvette, *Jour. Biol. Chem.*, 1928, LXXX, 589.

[30] Barlow, O. W., and R. W. Whitehead, *Amer. Jour. Physiol.*, 1929, LXXXIX, 552.

[31] It is regretted that we did not determine the body length of our rats, since this measurement would have enabled us to compare the nutritive states of the rats, as suggested by Cowgill and Drabkin (*Amer. Jour. Physiol.*, 1927, LXXXI, 41).

with the weights of Rats I and II when fasting essentially the same length of time, *i.e.*, 16 days and 2 hours, and 15 days and 20 hours, respectively, we find that the loss is 35 per cent for Rat III, but 45 and 48 per cent for Rats I and II, respectively. One could assume from the rate of loss, therefore, that the total metabolism of Rats I and II was higher during

TABLE II

LENGTH OF LIFE AND CHANGES IN BODY WEIGHTS OF FEMALE RATS FASTING AT 26°C

Date	Rat I (213 days)[1]		Rat II (206 days)[1]		Rat III (215 days)[1]		Rat IV (228 days)[1]	
	Duration of fast	Body weight	Duration of fast	Body weight	Duration of fast	Body weight	Duration of fast	Body weight
1928	Days Hrs.	gm.	Days Hrs.	gm.	Days Hrs.	gm.	Days Hrs.	gm.
Feb. 25	— —	—	— —	—	— 0	227[2]	— —	—
Feb. 26	— —	—	— 0	217[2]	— —	—	— 0	233[2]
Feb. 27	— 0	211[3]	— —	—	— —	—	— —	—
Feb. 27	— 3	214	— 16	202	1 16	211	1 3	231
Feb. 29	2 2	192	2 15	183	3 15	197	3 2	200
Mar. 2	4 2	176	4 15	174	5 15	189	5 2	182
Mar. 4	6 2	167	6 15	163	7 15	179	7 2	158[4]
Mar. 6	8 2	157	8 14	153	9 14	171	— —	—
Mar. 8	10 2	151	10 15	144	11 15	164	— —	—
Mar. 10	12 1	140	12 14	132	13 14	156	— —	—
Mar. 12	14 3	131	14 16	121	15 16	148	— —	—
Mar. 13	— —	—	15 20	112[3]	— —	—	— —	—
Mar. 14	16 2	117	— —	—	17 14	142	— —	—
Mar. 15	17 6	106[3]	— —	—	— —	—	— —	—
Mar. 16	— —	—	— —	—	19 13	136	— —	—
Mar. 17	— —	—	— —	—	20 13	131	— —	—
Mar. 19	— —	—	— —	—	22 13	122	— —	—
Mar. 21	— —	—	— —	—	24 13	112	— —	—
Mar. 21	— —	—	— —	—	25 0	105[3]	— —	—

[1] Age at beginning of fast.

[2] Weight when food was taken away and fast began.

[3] Weight at death.

[4] Weight at death not secured. Died morning of March 6, after 9 days and 2 hours without food.

their fasting life than was the metabolism of Rat III during the same number of hours' fasting. Reference to the data in Table IV (page 193) shows that this is the case. Moreover, the activity records (see Table IV), which serve as a rough indication of the metabolism, demonstrate that Rat I at least was consistently more active than Rat III. Greater activity alone, however, does not explain the differences in weight loss, for on the

last day of life Rat II was most active and yet the total metabolism (measured over a relatively short period of 6 hours) was lowest on that day.

Rat IV died after 9 days and 2 hours of fasting, but unfortunately no weight at death was obtained. When this animal had been without food for 7 days, it had lost 32 per cent of its initial weight, as compared with Rat I, which lost 26 per cent in 8 days, Rat II which lost 25 per cent in 6 days and 15 hours, and Rat III which lost 21 per cent in 7 days and 15 hours.

TABLE III

LENGTH OF LIFE AND CHANGES IN BODY WEIGHTS OF FEMALE RATS FASTING AT 16°C

Date	Duration of fast		Rat V (172 days)[1]	Rat VI (173 days)[1]	Rat VII (172 days)[1]	Rat VIII (174 days)[1]
			Body weight	Body weight	Body weight	Body weight
1929	Days	Hrs.	gm.	gm.	gm.	gm.
Jan. 3	—	0	215	217	211	221
Jan. 5	—	0	214	210	205	210
Jan. 6	—	0	215[2]	213[2]	206[2]	215[2]
Jan. 7	—	20	204	203	195	200
Jan. 9	2	20	178	184	173	178
Jan. 10	3	20	174	181	169	170
Jan. 11	4	20	165	176	162	162
Jan. 12	5	20	155	171	157	147
Jan. 13	6	22	149	165	150	134[3]
Jan. 14	8	2	133	150	135	—
Jan. 15	9	2	124	148	130	—
Jan. 16	9	21	119[3]	—	119[3]	—
Jan. 19	12	20	—	120[3]	—	—

[1] Age at beginning of fast.
[2] Weight at 1 P.M., Jan. 6, when food was taken away and fast began.
[3] Weight at death.

Of the rats fasting at 16° C., the total losses in weight were 45, 44, 42, and 38 per cent, respectively. Leaving out of consideration Rats IV and VIII, which lived the shortest lengths of time, and Rat III, which lived for 25 days, we find that the average length of life of Rats V, VI, and VII at the low temperature was 11 days and the total loss in weight 44 per cent, whereas the average length of life of Rats I and II at the high temperature was 16½ days or 50 per cent longer and the average total loss in weight was 49 per cent. Rats I and II at the warm temperature therefore lost 5 per cent more in weight, and this extra draft upon body weight sufficed to maintain them for a longer period of fasting, although at a distinctly

higher temperature. The metabolism of the rats fasting at 16° C., however, was considerably greater than that of the rats fasting at 26° C., as can be seen by reference to Tables IV and V. Thus, the rats at 16° C. had a heat production per square meter of body surface per 24 hours of essentially 1200 calories. The rats at 26° C., Rats I and II, had a heat production on this same basis averaging more nearly 550 calories. In other words, the metabolism at the low environmental temperature was nearly two and one-half times greater than the metabolism at the higher temperature. Yet the percentage losses in body weight and the length of life at the two different temperature levels do not show this same ratio. It is to be regretted that metabolism measurements were not made later than the seventh day in the fasts at 16° C. That the metabolism throughout the entire fasts of the rats at 16° C. would have remained at a level two and one-half times greater than the metabolism of the rats fasting at 26° C. is improbable. Indeed, one can imagine that there was a considerable fall in the metabolism throughout the latter part of the fasts at 16° C., as the vitality of the animals decreased.

One might ask whether these data indicate that there is a relationship between the loss in body weight and the total metabolism. As a result of the cold temperature, for instance, there may have been a heavier draft upon the storage of carbohydrates, although against this supposition is the fact that the respiratory quotient is always low at the start of the fast (24 hours after withdrawal of food), indicating a very low glycogen storage. Analyses of the bodies of the dead animals were not made. However, post-mortem examinations of the rats, both those that fasted at 26° C. and those that fasted at 16° C., showed that there was no appreciable visible fat. It would have been helpful to have determined the water content of the bodies and to have attempted to establish a water balance. In the experiments at the higher temperature there was a greater vaporization of water from the body. That this was compensated by the amount of water drunk can be assumed, but is not proved. The tissues of the rats which fasted at 16° C. may have contained a larger percentage of water than the tissues of the rats fasting in the warmer atmosphere. Loss in body weight alone as an index of the tissue consumption of the rat, an animal extremely sensitive to environmental temperature,[22] is therefore only of general value in predicting the probable total metabolism. Hence there is no definite relationship between body weight changes and total metabolism.

[22] The sensitivity of the rat to environmental temperature is emphasized by the experiments of Houssay and Artundo (*Compt. Rend. Soc. Biol.*, 1929, C, 124 and 127).

Another question which might be raised is whether the main factor determining death from inanition is the loss in weight. As we have already seen, the animals at 26° C. lived longer than those at 16° C. and actually drew more heavily upon body tissue. If death were due solely to the depletion of body stores as a whole, one would expect that the percentage loss in body tissue at death would be as great at 16° C. as at 26° C. Thus, one might assume that death would occur when the body weight loss amounted to 50 per cent. At the higher temperature it would take longer for an animal to lose half its weight than at the lower temperature. The rats at the cold temperature, however, died before this percentage loss occurred. They were, to be sure, living on a higher metabolic plane than were the rats at 26° C., but even the increased metabolism was not sufficient to use up their body substance so that they experienced as great a percentage loss in weight at death as did the rats at 26° C. Loss in weight alone cannot, therefore, be considered as the controlling factor in death.

Metabolism of Rats During Prolonged Fasting

Pembrey and Spriggs,[33] with the Haldane modification of the Pettenkofer method, measured the respiratory exchange of a rat (No. III) which fasted a total of 70 hours. Hári,[34] in his precise observations with his respiration calorimeter on the influence of carbohydrate upon heat production, reports metabolism measurements made upon rats fasting 24, 48, and 72 hours preceding the carbohydrate experiments. Since the environmental temperature during Pembrey's experiments was 19° C. and during Hári's experiments was 21° C., direct comparison of their results with our data obtained at 16° C. and 26° C. is not possible.

Mitchell and Carman,[35] in studying the effect of vitamin B upon the basal metabolism of rats, report among their data the results of an experiment with a rat which fasted for 53 hours. The rat was a male, 189 days old, weighing 273 grams. It was studied in the Haldane form of apparatus, at an environmental temperature between 28° and 31° C. In a period of measurement lasting 24 minutes, the respiratory quotient was found to be 0.75 and the heat production per 100 grams of body weight per day 9.4 calories (18.8 calories per 200 grams per day). The authors do not discuss this experiment, as they were not studying fasting *per se*. For their rats which fasted from 6 to 9 days at a temperature of about 30° C. Giaja and Males[36] report the basal heat production expressed in calories per

[33] Pembrey, M. S., and E. I. Spriggs, *Jour. Physiol.*, 1904, XXXI, 320.
[34] Hári, P., *Biochem. Zeitschr.*, 1913, LIII, 116.
[35] Mitchell, H. H., and G. G. Carman, *Amer. Jour. Physiol.*, 1926, LXXVI, 390.
[36] Giaja, J., and B. Males, *Compt. Rend. Soc. Biol.*, 1926, XCIV, 226.

kilogram of body weight per hour. They conclude from their data that only immediately before death (about 24 hours) does the basal metabolism begin to decrease and that there is a tendency for an increase in the middle of the fast. Since the metabolism during exposure to severe cold was also measured during these fasts, the results for the basal metabolism do not lend themselves to a study of fasting as the sole factor influencing the metabolism. Wesson[37] reports the heat production per kilogram of body weight per hour of rats fasting 72 and 96 hours, but does not enter into any discussion of the metabolism data as they were not important for his general thesis. In so far, therefore, as we have been able to ascertain from our compilation of the literature on the metabolism of rats (a compilation which, unfortunately, we cannot guarantee to be absolutely complete, owing to the difficulties encountered in tracing the literature), there is practically no information available regarding the metabolism of rats during prolonged fasting.

Method of presenting results. The metabolism of our rats fasting at 26° C. was determined in the multiple-chamber respiration apparatus[38] on every second day throughout the fasts, but the metabolism of the rats fasting at 16° C. was measured only twice, that is, at the end of 22 hours of fasting and near the end of the seventh day. The results of these observations are recorded in Tables IV and V. Each oxygen value represents the average of several well-agreeing periods of measurement, usually three each day, occasionally two. The length of the period was determined by the time required by the rat to absorb a definite volume of oxygen. At the beginning of the fast each period lasted for at least one hour or one and one-half hours, but toward the end of the fast, when oxygen was used at a slower rate, the periods were from three to four hours long.

The heat production has been calculated from the measured oxygen consumption, which has been considered to have a caloric value of 4.702 calories per liter, on the assumption that the fasting respiratory quotient is 0.72. (See page 179) For purposes of discussion, the oxygen consumption has been computed per 200 grams of rat per 6 hours and the heat production has been computed both per 200 grams of rat per 24 hours and per square meter of body surface per 24 hours. In referring the heat production to the surface area, the problem again came up as to the best method of computing the body surface. Profiting from our experience in our earlier experiments with rats,[39] in which we erred at first by em-

[37] Wesson, L. G., *Jour. Biol. Chem.*, 1927, LXXIII, 505.
[38] Benedict, F. G., *This Journal*, 1930, III, p. 161.
[39] Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, 360.

ploying a surface area formula including the incorrect constant furnished by Carman and Mitchell,[40] we used for these fasting experiments the formula $S = 9.1 \times w^{2/3}$. In this formula the factor 9.1 is the constant employed by Rubner[41] and confirmed by Giaja.[42] In addition, we also based our calculations upon the formula recently published by Lee[43] in which $S = 12.54 \times w^{0.60}$. Without entering into a discussion of the relative merits of these two formulas, we can state that the results for the heat production per unit of area on these two bases are essentially alike, being in general slightly higher with the Lee formula. As the fast progresses and the metabolism becomes lower per square meter of body surface, the results approach each other and the difference is insignificant at the end of the fast. We have the highest regard for the accurate work of Lee and his proposed formula for surface area, but since the Rubner constant of 9.1 has been used for many years and is more generally known, we shall, in our discussion of the heat production per square meter of body surface, consider only the calculations based upon the Rubner constant.

To aid in interpreting the data, we have indicated in Tables IV and V the relative activity of the rats during each period of measurement, expressed as the percentage of time that the animal was active during the actual period of the metabolism measurement. These estimates of relative activity are based upon an analysis of the kymograph tracings. From the irregular, broken portions of the kymograph tracing and from the time record drawn at the bottom of the tracing is computed the number of minutes during which the rat was active. Only that part of the tracing secured while the oxygen consumption was actually being measured is included in making this analysis. From the estimated minutes of activity and the total time of measurement is calculated the percentage of time during which the animal was active.

The sensitivity of the activity recording device is determined by noting the degree of deflection of the writing point when a 20-gram weight is dropped from a height of 10 centimeters upon the animal cage. This test is made at the beginning of the experiment and again at the end, and a kymograph record of the sensitivity is obtained when the final test is made. When calcium chloride is used inside the chamber, the tray containing the calcium chloride is placed on top of the animal cage, at the beginning of the experiment, the rubber bands are adjusted, and the

[40] Carman, G. G., and H. H. Mitchell, *Amer. Jour. Physiol.*, 1926, LXXVI, 380.
[41] Rubner, M., Die Gesetze des Energieverbrauchs bei der Ernährung, Leipzig, 1902, 281.
[42] Giaja, J., *Ann. de Physiol.*, 1925, I, 607.
[43] Lee, M. O., *Amer. Jour. Physiol.*, 1929, LXXXIX, 24.

TABLE IV

METABOLISM OF FEMALE RATS FASTING AT 26°C. (MEASURED AT 28°C.)

Rat No.	Date	Body weight	Duration of fast		Oxygen consumed per 6 hours		Heat produced per 24 hours			Activity
					Total	Per 200 gm.	Total	Per 200 gm.	Per sq. m.	
	1928	gm.	days	hrs.	cc.	cc.	cal.	cal.	cal.	p. ct.
I	Feb. 27	214	—	3	1174	1097	22.1	20.6	677	—
	Feb. 29	192	2	2	1001	1043	18.8	19.6	621	15
	Mar. 2	176	4	2	896	1019	16.9	19.2	589	10
	Mar. 4	167	6	2	785	940	14.8	17.7	535	12
	Mar. 6	157	8	2	797	1015	15.0	19.1	566	11
	Mar. 8	151	10	2	735	973	13.8	18.3	536	7
	Mar. 10	140	12	1	692	988	13.0	18.6	531	11
	Mar. 12	131	14	3	710	1084	13.4	20.4	568	16
	Mar. 14	117	16	2	566	967	10.6	18.2	488	14
II	Feb. 27	202	—	16	1207	1195	22.7	22.5	725	14
	Feb. 29	183	2	15	972	1062	18.3	20.0	624	15
	Mar. 2	174	4	15	821	944	15.4	17.8	544	12
	Mar. 4	163	6	15	699	857	13.1	16.1	483	7
	Mar. 6	153	8	14	666	870	12.5	16.4	482	7
	Mar. 8	144	10	15	634	881	11.9	16.6	477	—
	Mar. 10	132	12	14	624	946	11.7	17.8	497	2
	Mar. 12	121	14	16	507	837	9.5	15.7	428	24
III	Feb. 27	211	1	16	1039	985	19.5	18.5	605	14
	Feb. 29	197	3	15	897	911	16.9	17.1	548	20
	Mar. 2	189	5	15	785	831	14.8	15.6	492	12
	Mar. 4	179	7	15	660	737	12.4	13.9	430	2
	Mar. 6	171	9	14	652	763	12.3	14.3	438	8
	Mar. 8	164	11	15	663	808	12.5	15.2	457	3
	Mar. 10	156	13	14	608	779	11.4	14.7	433	4
	Mar. 12	148	15	16	627	847	11.8	15.9	462	7
	Mar. 14	142	17	14	614	864	11.5	16.3	464	10
	Mar. 16	136	19	13	585	860	11.0	16.2	457	7
	Mar. 17	131	20	13	583	890	11.0	16.7	464	10
	Mar. 19	122	22	13	532	872	10.0	16.4	447	8
	Mar. 21	112	24	13	469	837	8.8	15.7	418	14
IV	Feb. 27	231	1	3	1150	996	21.6	18.7	631	21
	Feb. 29	200	3	2	908	908	17.1	17.1	549	19
	Mar. 2	182	5	2	878	965	16.5	18.1	566	15
	Mar. 4	158	7	2	787	997	14.8	18.8	556	21

sensitivity of the activity recording device is tested by tapping the edge of the cage. At the close of the experiment the calcium-chloride tray is removed and a 50-gram weight is dropped from a height of 10 centimeters upon the cage, with the kymograph in place.

Oxygen Consumption

The total oxygen consumption of the rats which fasted at 26° C. decreased as the fast progressed. This is to be expected because of the continually diminishing body weights of the rats. The oxygen consumption per 200 grams of rat per 6 hours (assuming 200 grams as representing the average weight of an adult rat) shows an altogether different picture. Thus, certainly with Rat I, the oxygen consumption on this basis is essentially uniform throughout the entire fast of 16 days, the minimum occurring on the sixth day, the maximum at the third hour, and a value only slightly below the maximum occurring on the fourteenth day. With this particular animal, therefore, the metabolism may be considered to be constant per unit of weight, irrespective of the length of the fast. With Rat II the situation is different. There are high values for the oxygen consumption per 200 grams on the first two days, and the minimum value occurs on the last day. Moreover, the level of the oxygen consumption of this rat throughout the fast is lower than that of Rat I, being nearer 900 cc. per 200 grams of body weight per 6 hours after the first two days, whereas with Rat I it is nearer 1000 cc. Rat III, which underwent the longest fast, showed likewise a fairly wide range between the maximum and minimum oxygen consumption per unit of weight. The minimum value appears at the end of 7 days and 15 hours and yet after 3 days and 15 hours there is a tendency toward constancy except for a slight rise near the end of the fast. The general level of the oxygen consumption of this rat is about 850 cc. per 200 grams per 6 hours. Although the data for Rat IV are not so numerous as for the other rats in this group, they indicate that through the seventh day of fasting the metabolism was essentially constant at a level of about 970 cc. In general, although the total oxygen consumption of the rats fasting at 26° C. fell off markedly as the fast progressed, the oxygen consumption per 200 grams of body weight per 6 hours, after an initial fall during the first one or two days, tended to remain at a constant level of not far from 900 to 1000 cc.

Of the animals which fasted at 16° C., Rat V was unusually active but the other three rats were, on the average, not any more active than the rats studied at 26° C. The total oxygen consumption, however, of the four rats at 16° C. averaged at the 22nd hour of fasting not far from 2100 cc. per

6 hours or nearly twice as much as that of the rats at 26° C. when fasting about the same length of time. Even after seven days of fasting, the total oxygen consumption is high, averaging about 1700 cc. or considerably over twice that of the rats maintained at the warmer temperature. In other words, the level of the total metabolism of fasting rats is much lower at the warm temperature than at the cold temperature, even when the amount of activity is in general the same.

Both groups show a decrease in total metabolism with continued inanition, but the fall is much more pronounced at the high than at the low temperature. Thus, the average total oxygen consumption of rats fasting at 26° C. was not far from 1100 cc. per 6 hours at the beginning of the

TABLE V
METABOLISM OF FEMALE RATS FASTING AT 16°C. (MEASURED AT 16°C.)

Rat No.	Date	Body weight	Duration of fast		Oxygen consumed per 6 hours		Heat produced per 24 hours			Activity
					Total	Per 200 gm.	Total	Per 200 gm.	Per sq. m.	
	1929	gm.	days	hrs.	cc.	cc.	cal.	cal.	cal.	p. ct.
V	Jan. 7	204	—	22	2079	2038	39.2	38.4	1244	32
	Jan. 13	149	6	22	1779	2388	33.5	45.0	1309	50
VI	Jan. 7	203	—	22	2069	2038	39.0	38.4	1242	5
	Jan. 13	165	6	22	1700	2061	32.1	38.9	1172	15
VII	Jan. 7	195	—	22	2081	2135	39.2	40.2	1281	6
	Jan. 13	150	6	22	1626	2168	30.7	40.9	1195	20
VIII	Jan. 7	200	—	22	2127	2127	40.1	40.1	1289	17

fasts. At the end of seven days it was more nearly 700 cc., representing a fall of 400 cc. or 36 per cent. With the rats fasting at 16° C., on the other hand, the total oxygen consumption was practically 2100 cc. at the start and fell to 1700 cc. at the end of 7 days, a decrease of 19 per cent. Hence the percentage decrease in the total metabolism as a result of the fasting was much less at the low temperature than at the high.

The oxygen consumption per unit of weight of the rats at 16° C. did not decrease with the progress of the fast. In this respect both groups of animals show the same picture. Indeed, for the three rats at the low temperature for which the comparison is available, the oxygen consumption per unit of weight is actually somewhat higher at the end of the seventh day of fasting than it is at the beginning.

Heat Production

Total heat production. The total heat production follows the same course as the total oxygen consumption, that is, decreasing distinctly as the fast progresses. Indeed, with three of the rats at 26° C. the total heat production on the last day of the fast is less than half that on the first day, for whereas at the start the total heat production is not far from 20 calories per 24 hours, it falls to as low as 9 calories just prior to death. The total heat production of the rats at 16° C., like the total oxygen consumption, is at a higher level than that of the rats at 26° C., but decreases only about 18 per cent at the seventh day of fasting as compared with the decrease of about 35 or 40 per cent with the other group of rats, when fasting the same length of time.

Heat production per 200 grams of body weight per 24 hours. The heat production per unit of weight shows the same picture for the rats fasting at 26° C. as does the oxygen consumption per unit of weight. Thus, although there is a tendency for the heat production per 200 grams of weight to decrease somewhat, it remains more nearly at a constant level than does the total heat production.[44] With the rats at 16° C., however, the heat production on this basis does not tend to decrease but actually is slightly higher on the seventh day. Indeed, with Rat V there is a pronounced increase of 17 per cent. A possible explanation of the sustained high metabolism at 16° C. is that the rats at the low temperature were somewhat more active than the rats at the warmer temperature.

Heat production per square meter of body surface per 24 hours. When the heat production is computed per square meter of body surface, using the formula $S = 9.1 \times w^{2/3}$ (see page 192), we find that among the rats fasting at 26°C. the metabolism at the start averages under 700 calories, the highest value being 725 calories at the fifteenth hour with Rat II. At this time, to be sure, the activity was fairly great. In all cases, however, the tendency is for the metabolism on this basis to fall off distinctly as the fast progresses. Toward the end of the fast, when the undernutrition is pronounced and the vital activity of the rat is low, the values are extraordinarily small, the minimum being 418 calories at the end of the long fast of Rat III. Hence it is possible for the metabolism of the albino rat to approach a level as low as 400 or 450 calories per square meter of body surface per 24 hours during prolonged fasting at 26° C. At the low temperature

[44] Rubner (Die Gesetze des Energieverbrauchs bei der Ernährung, Leipzig, 1902, 278), in his classic study with the fasting guinea pig, was perhaps the first to point out most strikingly that, although the total heat production falls off pronouncedly during fasting, the heat production per kilogram of body weight remains on the average practically unchanged.

the heat production per unit of surface area on the first day of fasting is considerably greater than that at the warm temperature. It averages 1260 calories at the start and falls only imperceptibly in seven days of fasting, again in striking contrast to the picture shown by the group at the warm temperature. The high metabolism at 16°C., averaging 1260 calories at the twenty-second hour, may be compared with the results obtained by Benedict and MacLeod[45] with rats living at this same temperature. From their average curve for the heat production of rats referred to environmental temperature we find that their series of rats, 16 hours after food, would produce at 16°C. more nearly 1500 calories per square meter of body surface per 24 hours. As they have pointed out, however, there is a considerable difference in the level of the metabolism of the individual rats at the different temperatures. Yet the percentage effect upon metabolism of each degree change in environmental temperature is much the same with all rats. At least one of the rats in their chart has a metabolism at 16°C. of 1300 calories, a value more in line with the data obtained in our fasting series. The rats that fasted at 16°C. had, to be sure, lost 4 grams each during the period of adjustment to the new laboratory environment, and yet their lower initial heat production (1260 calories) as compared with the heat production of the Benedict-MacLeod rats (1500 calories) can hardly be explained by the assumption that they had reached a stage of incipient undernutrition before their fasting period began.

The Basal Metabolism of the Adult, Female, Albino Rat

Of the various animals commonly studied in laboratories to determine the effect upon metabolism of such factors as activity, drugs, endocrine glands, and special diets, none is perhaps more frequently employed than is the albino rat. To the student of comparative physiology one of the most interesting features in such investigations with albino rats is the comparison of its heat production with that of larger and smaller animals of different species. The most generally accepted method of making this comparison is to refer the metabolism to some unit of surface area. For purposes of discussion we have computed the heat production per square meter of body surface, employing the formula $S = 9.1 \times w^{2/3}$ (see page 192). A comparison of the metabolism of animals of different species is preferably based upon observations made at the so-called "critical temperature" of the animals, that is, at that temperature of the environment at which the

[45] Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, fig. 1, 369.

metabolism is essentially minimum. With rats this critical temperature was found to be not far from 28°C. The original experiments of Rubner in which he formulated his "body surface law" did not lay stress upon environmental temperature. Hence in using the minimum metabolism of animals as the basis for the comparison, we are perhaps in error. Yet, in view of the pronounced influence of environmental temperature upon an animal's metabolism, an influence which Rubner was the first to point out in a striking way with the guinea pig and also with the dog, it would seem that a comparison of the metabolism of different animals could not be made on any better basis than that of the metabolism obtaining at the critical temperature.

The results obtained with our rats that fasted at 16°C. may not be used in a study of comparative physiology, as 16°C. is not the critical temperature for the rat. Moreover, the data secured toward the end of the fasts at 26°C. also may not be used, since the comparison of the basal metabolism of different animals requires that the metabolism should have been determined under normal conditions of nutrition and in the post-absorptive state. The error is frequently made of basing this comparison upon animals not in the same state of nutrition, and we wish to caution against this. For such comparison, therefore, we have available in our fasting studies only the data secured with rats in the first days of fasting at an environmental temperature of 26°C.

The initial heat production per unit of surface area of our female rats, fasting at 26°C., is low, from 600 to 700 calories per square meter of body surface per 24 hours. Even but three hours after the removal of food Rat I has a heat production on this basis of under 700 calories. It is unfortunate that in the research of Benedict and MacLeod[46] the number of female rats measured at the average age of these rats, about 200 days, was small. Their curve for the heat production per square meter of body surface per 24 hours of female rats at 28.9°C. shows, however, that at the age of 7½ months, or about 225 days, the metabolism is not far from 700 calories, a value quite in line with the observations secured on our rats fasting at 26°C.

In attempting to establish an average value for the basal metabolism of an animal we would emphasize, as did Verzár,[47] that the most reliable measurements from which such an average or standard may be derived are secured not in periods of only 5 or 20 minutes' duration, as were those

[46] Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, fig. 2, 381.
[47] Verzár, F., *Arch. f. d. ges. Physiol.*, 1929, CCXXII, 717.

of Giaja,[48] Mitchell and Carman,[49] Bijlsma,[50] Wu and Chen,[51] and Nothhaas,[52] but in periods covering several hours. Indeed, we do not approve of measuring the *basal* metabolism in short periods of only a few minutes, for even if the animal is not active during this time, there may be an after-effect of previous activity holding over into the period of measurement, and if the technique calls for gas analysis, it is difficult to secure a representative sample in such a short time.

Resistance of Rats to Prolonged Fasting

The most striking feature of our study is the fact that the albino rat can withstand fasting for such a long period. Indeed, one rat at 26°C. lived for 25 days which, so far as we are aware, is quite outside the experience in any other laboratory. Even the rats that fasted at 16°C. and that consequently had a much greater heat production, lived a surprisingly long time, in fact, much beyond the conjectured two or three days cited by Morgulis.[53] It is possible that the length of life of these rats during fasting may be in large part accounted for by the fact that they were free from structural defects and from internal parasites, as shown by actual autopsies. Likewise they had no external parasites. In other words, we were dealing with normal, healthy albino rats. Contrary to the supposition, therefore, that a small animal such as the rat succumbs quickly to fasting, our evidence proves that the adult albino rat can withstand fasting for from 9 to 25 days at 26°C., and that even when the effect of a cold environmental temperature, 16°C., is superimposed upon the effect of fasting, the rat may live for at least 7 to 12 days.

Summary

The metabolism of female albino rats (average age about 200 days) was studied during fasting prolonged until the occurrence of death. Four rats were maintained at an environmental temperature of 26°C. and four other rats at a temperature of 16°C. A multiple-chamber, closed-circuit respiration apparatus was employed, and the oxygen consumption was directly measured in periods of from one to four hours in length.

The rats fasting at 26°C. lived on the average for 16½ days and lost 49 per cent of their initial body weight. The animals fasting at 16°C. survived

[48] Giaja, J., *Ann. de Physiol.*, 1925, I, 596.
[49] Mitchell, H. H., and G. G. Carman, *Amer. Jour. Physiol.*, 1926, LXXVI, 385.
[50] Bijlsma, U. G., *Verslagen en Mededeelingen betreffende de Volksgesondheid*, August, 1926.
[51] Wu, H., and T. T. Chen, *Chinese Jour. Physiol.*, 1929, III, 307 and 315.
[52] Nothhaas, R., *Arch. f. d. ges. Physiol.*, 1929, CCXXI, 763.
[53] Morgulis, S., Fasting and Undernutrition, New York, 1923, 93.

on the average a shorter time, 11 days, and lost somewhat less weight, 44 per cent. The longest fast was at 26°C. and lasted 25 days.

The total metabolism of the rats fasting at 26°C. (but measured at 28°C.) was at a lower level than was that of the group fasting and measured at 16°C., but the decrease in the total metabolism as the fast progressed was greater, amounting on the average to 36 per cent at the end of the seventh day as compared with a decrease of 19 per cent noted with the rats at 16°C., fasting the same length of time. The metabolism per unit of weight decreased much less markedly than the total metabolism, in the case of both groups, and tended to become fairly constant. Per square meter of body surface the heat production of the group at 26°C. decreased distinctly as the fast progressed, from an initial average of 700 calories to a level at the end of the fast as low as 400 or 450 calories per 24 hours. With the group at 16°C. the heat production on this basis averaged 1260 calories at the 22nd hour and fell only imperceptibly in 7 days.

The basal metabolism of these adult female rats fasting at 26°C. (but measured at 28°C.) and about 24 hours after food was from 600 to 700 calories per square meter of body surface per 24 hours.

The length of survival of these fasting rats refutes the supposition that small animals succumb quickly to fasting.

STUDIES IN CARBOHYDRATE METABOLISM FOLLOWING GUANIDINE DEGLYCOGENATION

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Introduction

STUDIES of guanidine hypoglycemia have shown that the fall in blood sugar does not take place until the liver glycogen has been depleted (Bodo and Marks, 1928, Bischoff, Sahyun and Long, 1929). A pronounced decrease in muscle glycogen is brought about along with the depletion of the liver glycogen. In the present work advantage is taken of this property of guanidine derivatives to study carbohydrate metabolism after deglycogenation. The guanidine derivatives do not react solely upon the glycogen reserves. The liver is generally poisoned as shown by a yellow atrophy and disability to carry on deaminization. The kidneys are also damaged as shown by pathological examination and rise in urea nitrogen. In investigating the hypoglycemic activity of a number of guanidine derivatives we found (Bischoff, Sahyun and Long, 1929) instances in which the animal was able to utilize large amounts of glucose following the action of the guanidine compounds.

In the present study we have investigated the mechanism of the utilization of exogenous glucose by the rabbit, which had suffered total hepatic deglycogenation and partial or total muscle deglycogenation, through guanidine poisoning. It was essential to establish whether the liver glycogen had been merely depleted or whether this organ had lost the power to synthesize glycogen, when supplied with glucose. In the experiments of Bodo and Marks (1928) muscle glycogen values are not reported with liver glycogen values. The work of Staub (1927), Bodo and Marks (1928) and Bischoff (1929) showed that synthalin and similar compounds break down the muscle glycogen, but the extent and the nature, whether mere depletion or production of inability to synthesize glycogen, had still to be determined. The answers to these questions are of interest not only in the study of guanidine hypoglycemia but also in the study of carbohydrate metabolism in general. The main portion of this paper is concerned with a problem of fundamental importance, namely, the relation of the utilization of glucose as regulated by insulin and adrenalin to the intermediate formation of glycogen in a deglycogenated animal.

Experimental Procedure

The rabbit has found disfavor in studies of carbohydrate metabolism because of the great variation in its glycogen reserves. Goldblatt (1929) has recently shown that the glycogen content of litter mates is not subject to great variation. We have followed his plan of using litter mates for each series of our experiments. Our working plan was as follows:

1. A group of litter mates was fasted 24 to 48 hours.
2. One litter mate was killed for liver and muscle glycogen.
3. Three remaining litter mates were given a hypoglycemic dose of diguanidino-penta-methylene, or of synthalin.
4. Dosed rabbits were bled every 2 hours to establish onset of hypoglycemia.
5. At onset of hypoglycemia, the three rabbits were given a glucose tolerance, (a) one rabbit serving as a control, (b) one rabbit receiving insulin, (c) one rabbit receiving adrenalin.
6. Blood sugars and lactic acids were determined every hour after glucose tolerance from 4 to 8 hours.
7. Rabbits were killed, and muscle and liver analyzed for glycogen.

In the first experiments the glucose was given orally. Analyses of the stomach contents indicated that 90 per cent absorption took place during the course of the experiment, when small doses of glucose were given. In order to raise the blood sugar appreciably, large doses of glucose had to be given by mouth. The extent to which glucose absorption took place was doubtful in these experiments. In the last experiments the glucose was therefore given subcutaneously.

Because of the great variation in the tolerance of rabbits to hypoglycemia-producing guanidine derivatives, the correct dosage was a factor of no little importance to the success of the experiment. Former experience with di-guanidino-penta-methylene and deca-methylene (synthalin) had shown that the minimum hypoglycemic dose for these compounds was 60 and 4 mg. of base per kilo., given intravenously. While several groups of litter mates responded satisfactorily to these doses, others showed only a transient hypoglycemia or no hypoglycemia at all. In two series of experiments the dose of di-guanidino-penta-methylene was raised to 70 mg. per kilo. In several synthalin series, the dose was repeated when hypoglycemia failed to develop within 24 hours. The uniformity of litter mates in regard to physiological response was admirably demonstrated in these experiments. While different series showed a tremendous variation in response to the guanidine derivatives, individuals of the same litter showed parallel blood sugar values with but two exceptions. In one of the

TABLE I

SHOWING EFFECT OF INSULIN UPON GLUCOSE ADMINISTRATION FOLLOWING DIGUANIDINO PENTA-METHYLENE HYPOGLYCEMIA

Exp.	Wt. of rabbit	Time required to produce hypoglycemia	Treatment at hypoglycemia and subsequently. (all doses per kilo.)	Blood sugar in mg. per 100 cc.							Glycogen at death in mg. per 100 g.	
				Initial	At hypoglycemia	After administration of glucose						
						1.5 hr.	2 hr.	3 hr.	4.5 hr.	6 hr.	liver	muscle
	kg.	hrs.										
I	1.8	24	2.3 gm. glucose per os	85	50	74	91	125	118		7	0
II a	2.7	7	2.0 gm. glucose per os	94	55	125	131	125	109		10	60
II b	2.6	7	2.0 gm. glucose per os plus 6 u. insulin intrav.	98	63	77	77	55	59		10	63
III a	2.5	20	3.0 gm. glucose per os repeat 3rd hr.	87	56	125	160	157	160	139	8	140
III b	2.4	20	3.0 gm. glucose per os repeat 3rd hr. 6 u. insulin intrav.	91	49	60	60	73	52	64	10	240
III c	2.6	20	3.0 gm. glucose per os repeat 3rd hr. 6 u. insulin intrav. 0.4 mg. adrenalin subcu.	87	52	88	108	140	133	130	7	43

TABLE II

EFFECT OF INSULIN AND OF ADRENALIN UPON GLUCOSE UTILIZATION FOLLOWING SYNTHALIN HYPOGLYCEMIA.

Exp.	Wt. of rabbit kg.	Hours required to produce hypoglycemia	Treatment at hypoglycemia and subsequently (all doses per kilo.)	Blood findings in mg. per 100 cc.								Glycogen at death in mg. per 100 gm.		Urine sugar
				Substance	Initial	At hypoglycemia	After giving glucose 1.5 hr.	2 hr.	3 hr.	4.5 hr.	6 hr.	liver	muscle	
I	3.0	5	3.0 gm glucose[1] Repeat 2nd hr.[2]	glucose lactic acid	82 27	48 300	43 420		276 420			tr	7	0
II a	3.2	6	3.0 gm. glucose[1] 0.35 mg. adrenalin	glucose lactic acid	100 46	75 63	137 140		208 170	232 104		tr	36	0
II b	2.9	6	3.0 gm. glucose[1] 13 u. insulin	glucose lactic acid	87 25	61 140	60 150		58 90	60 73		tr	133	0
II c	2.8	6	3.0 glucose[1]	glucose lactic acid	87 54	76 115	96 160		100 73	138 73		83	280	
III a	2.3	4.5	10.0 gm. glucose[1]	amino acid N glucose lactic acid	10.4 77	13.1 47 125		121	248 128	17.2 276 117		0	45	0
III b	2.1	4.5	10.0 gm. glucose[1] 5.0 u insulin	amino acid N glucose lactic acid	11.0 87	15.0 38 128		90 134	94 133	15.0 83 122		0	15	0
III c	2.3	4.5	10.0 gm. glucose[1] 0.4 mg. adrenalin	amino acid N glucose lactic acid	11.1 83	14.0 61 100		272 93	338	17.2 400 138		0	19	0
IV a	3.8	32	5.0 gm. glucose[3]	glucose	85	95		526	526	526		68	19.5	
IV b	3.5	30	5.0 gm. glucose[3] 14 u. insulin	glucose	77	46		292	192	142		21	70	0

TABLE II (*continued*)

TABLE II (*continued*)

IV c	3.9	30	5.0 gm. glucose[2] 0.25 mg. adrenalin	glucose	93	48		266	356	320*		0	0	0
Va	1.5	13	5.0 gm. glucose[2]	glucose lactic acid	93 41	42 83	620		696 109	552 95		0	0	29
Vb	1.5	13	5.0 gm. glucose[2] 25 u. insulin 2 doses	glucose lactic acid	79 24	53 79	652 84		240 120	74 109		0	110	0
Vc	1.3	13	5.0 gm. glucose[2] 0.8 mg. adrenalin	glucose lactic acid	93 22	67 83	348 80		377 122	274* 130		0	0	0
VIa	3.5	9	3.0 gm. glucose[2]	amino acid N glucose lactic acid	13.7 116 32	64 125	222		242	 210 148	48.5 160 158	0	0	0
VIb	3.2	9	3.0 gm. glucose[2] 1.5 gm. glucose 4th hr. 25 u. insulin 2 doses	amino acid N glucose lactic acid	11.0 127 52	65 113	262		168	 127 148	38 132 156	0	10	0
VIc	3.0	10	3.0 gm. glucose[2] 0.33 mg. adrenalin	amino acid N glucose lactic acid	 120 47	74 100	348		444	 516 178	38 500 174	0	9	0
VIIa	1.8	7	3.0 gm. glucose[2] 4.0 mg. synthalin[2]	glucose lactic acid	109	50 171	240		268 234	222 159	164 168	0	13	0
VIIb	1.6	7	12.0 gm. glucose 4 doses[2] 24 u. insulin 4.0 mg. synthalin[2]	glucose lactic acid	100 25	50 144	143 173		124 237	228 224	182 222	0	153**	
VIIc	1.6	7	3.0 gm. glucose[2] 4.0 mg. synthalin[2] 0.6 mg. adrenalin	glucose lactic acid	105	67 113	350 185		416	374 261	250 207	0	0	0

* The animal was dying.

** The heart, weighing 7.0 gm., contained 42.8 mg. glycogen.

[1] per os. [2] subcutaneously. [3] intraperitoneally.

Insulin was given intravenously.

Adrenalin was given subcutaneously.

series, hypoglycemia in one animal began two hours after hypoglycemia developed in its litter mates. We have not made any deductions from results of experiments in which the behavior of the litter mates was not uniform, *viz*, series II and IV of the synthalin group. In the experiments II and IV, the livers of two rabbits had obviously not lost their power to synthesize glycogen. In experiment IV, the blood sugar had returned to normal before glucose was given.

Glucose was determined by the method of Folin and Wu, glycogen by that of Pflüger and blood lactic acid by that of Mendel (1925). The lactic acid method, in our hands, did not show the accuracy claimed for it by the author, and the values cannot be regarded as absolute. In determining glycogen, the animal was stunned by a blow upon the head, the liver and hind leg muscles being instantly removed. The time elapsing between the excising, and plunging into the caustic was less than 30 seconds. The data will be found in Tables I and II.

The liver glycogen. The glycogen content of the liver and muscle following the action of the guanidine compound was determined for twenty-six rabbits. These animals had all been subjected to a glucose tolerance for a period of hours before being killed. Of these twenty-six rabbits the liver of twenty-three contained less than 0.01 per cent glycogen. One liver analyzed 0.02 per cent glycogen. Two livers analyzed 0.07 and 0.08 per cent glycogen. The blood sugar determinations for these rabbits showed that twenty-three of the twenty-six rabbits which had the very low glycogen contents were below 60 mg. per 100 cc. at the time the glucose was given. The two rabbits which showed 0.07 to 0.08 per cent glycogen in the liver had blood sugars of 76 and 95, when the glucose was given. In one of these animals the blood sugar had not fallen at any time; in the other it had fallen to 59 but returned to 95, at the time the glucose was given. Of the twenty-six rabbits which received the glucose tolerance following guanidine hypoglycemia, eight received insulin simultaneously and seven adrenalin. Whereas both insulin and adrenalin would, with normal rabbits under the same condition, show an increase in liver glycogen (Sahyun and Luck, 1929, Goldblatt, 1929, Collazo, Händel and Rubino 1924), the rabbits under guanidine treatment showed a depletion of liver glycogen. The liver glycogen of five rabbits which were not given glucose was also determined at the onset of hypoglycemia. The analyses were all less than 0.01 per cent. *From these data we feel justified in concluding that when the guanidine derivative has lowered the blood sugar to 60 mg. per 100 cc. or below, the hepatic glycogen reserves are depleted and hepatic glycogenesis cannot take place even though the animal be given a hyperglycemic level of*

glucose for a period of four to six hours. Bodo and Marks (1928) found liver glycogen values of 0.02 to 0.06 for rabbits killed in synthalin hypoglycemia. For one animal in which the synthalin did not produce hypoglycemia, the value found was 0.08 per cent. This value checks our value for the rabbit which did not show hypoglycemia and the rabbit which had recovered. The slightly higher glycogen values obtained by Bodo and Marks for rabbits in synthalin hypoglycemia are undoubtedly due to a difference in experimental procedure. These authors gave considerably larger doses of guanidine compound than we did so that it was necessary to kill the animals before the 6th hour after dosing. None of our animals was killed before the 9th hour; two series were killed as late as the 36th hour. We attempted to give the smallest dose which would produce hypoglycemia. The liver glycogen of three rabbits, litter mates of several of the series given guanidine derivatives, was determined after fasting 24, 48, and 96 hours. The glycogen values were 3.9, 3.2, and 1.0 per cent.

Muscle Glycogen. While the liver glycogen is practically depleted in hypoglycemia produced by guanidine compounds, the muscles may contain measurable quantities of glycogen. We reported 33 and 60 mg. per 100 grams tissue in earlier experiments (Bischoff, Sahyun and Long, 1929). Five control experiments were run in the present series. The rabbits were killed at the onset of hypoglycemia, at the stage where they would have been given the glucose. None of these rabbits showed any liver glycogen. The muscle glycogen in mg. per 100 grams tissue was 0, 17, 23, 70, and 76. The rabbit which had no glycogen at the time of initial guanidine hypoglycemia was a litter mate of the rabbits in Experiment V of the synthalin series. The rabbits which showed 17 and 23 mg. were also litter mates. In Table III we have compared the glycogen contents of the muscle of

TABLE III

EFFECT OF INSULIN, GUANIDINES, AND ADRENALIN UPON THE GLYCOGEN CONTENT OF THE MUSCLE OF THE RABBIT

Investigators	Fasted 24 hours		At insulin hypoglycemia		At guanidine hypoglycemia		At adrenalin hyperglycemia (2 hrs.)	
	Extremes	Mean	Extremes	Mean	Extreme	Mean	Extreme	Mean
Goldblatt	60–430	228	80–250	180				
Sahyun and Luck	400–620	497	330–490	390			54–100	74
Bischoff and Long	270–440	335			0–76	37		

the rabbit fasted 24 hours, during insulin hypoglycemia and during guanidine hypoglycemia. The results of different investigators vary considerably as to the glycogen content of the rabbit fasted 24 hours. The interpretation of the results nevertheless offers no difficulty: *while the muscle glycogen is only slightly lowered at insulin hypoglycemia, it is more than three-fourths depleted at guanidine hypoglycemia.* It should be noted that the results of Goldblatt are for rabbits in insulin convulsion, while the results of Sahyun and Bischoff are at hypoglycemia prior to convulsion symptoms. In most of the experiments recorded in this paper, the animal had been subjected to a glucose tolerance prior to killing. There is a possibility therefore that some muscle glycogen may have been built up during the hyperglycemia induced by the glucose. Eight values of muscle glycogen for rabbits which were in a state of hyperglycemia (glucose tolerance) following the guanidine hypoglycemia were 0, 0, 0, 7, 13, 45, 60, and 140 mg. per 100 grams. The values for the eight rabbits which were given insulin under the identical experimental conditions were 10, 15, 63, 70, 110, 133, 153, and 240. The values for those which had received adrenalin were 0, 0, 0, 9, 19, 36, and 43. These glycogen values are recorded in ascending order. In order to appreciate the relative effects for each series of litter mates it is necessary to consult Table I and Table II. It is evident that the rabbits which had received adrenalin showed the lowest muscle glycogen, those that received insulin the highest, while the controls were intermediate. Since the muscle glycogen content is so much lower in these experiments than in the normal animals, the effect of adrenalin in lowering the muscle glycogen as reported by Sahyun and Luck (1929) and others, is not so clearly defined because the differences observed were within the range of the expected variation in the different animals. The action of the insulin was variable. In three cases it raised the muscle glycogen to 240, 153, and 130 mg. per 100 grams. In two other instances the muscle glycogens were practically nil, 10 and 15 mg. per 100.

Blood Sugar. In the seven series of experiments in which the action of insulin and adrenalin were studied following the depletion of the glycogen reserves, a weighed quantity of glucose was given each animal, orally, intraperitoneally, or subcutaneously, following hypoglycemia. The blood sugar curves of the controls (those receiving neither insulin nor adrenalin) show that sufficient glucose had been given in every case to produce a normal or hyperglycemic level. The blood sugar curve was decidedly lower in every instance for the rabbits receiving insulin. The blood sugar curve for the rabbits receiving adrenalin was higher in every case than that for the rabbits receiving insulin, but in two instances it was lower than that

of the controls. Both of the animals with the low sugar curves were prostrated at the end of the experiment, apparently about to expire, so that we have not felt justified in comparing the results with those of the litter mates or those of the rabbits in the other experiments. In the first experiments, the glucose was given orally. In the last experiments the glucose was given parenterally. It should be noted that in Experiment VI of the synthalin series, the rabbit that had received insulin was given 1.5 grams more glucose per kilo. than the other rabbits in the series. This added glucose was given to prevent a terminal hypoglycemia. The increase in glucose utilization by insulin is therefore greater than would be indicated by the blood sugar curves alone. It should be noted that in Experiment V of the insulin series, the control rabbit excreted 2.0 grams of sugar. Here again the increased utilization of the glucose by insulin is greater than is indicated by the blood sugar curves. In experiment VII, four times as much glucose was given to the rabbit which had received insulin as to the control rabbit.

The Glucose Tolerance. In order to make the blood sugar curves in the present series of experiments comparable with the curves that would have been obtained with normal animals, we have established the blood sugar curves for several normal rabbits upon a gradation of doses of glucose. The rabbit has a considerably higher renal threshold for glucose than man. Given 2.0 grams of glucose per kilo., the rabbit's blood sugar will not rise much above 150 mg. per 100 cc. Five grams per kilo. will produce a blood sugar curve a little above 200 mg. Ten grams per kilo. will produce a curve below 300 mg. It is necessary to give 20 grams per kilo. in order to produce a blood sugar level over 400 mg. when sugar in the urine will show simultaneously. Typical glucose tolerance curves for normal rabbits are given in Table IV.

In the present series of experiments, we were fortunate enough to have two rabbits which survived the dose of diguanidino-penta-methylene. These rabbits had been given a glucose tolerance dose of 3.0 grams glucose per kilo. at the time of guanidine hypoglycemia. The glucose tolerance test was repeated the day following recovery, the sugar curve being lower. If the blood sugar curves in the present series of experiments are compared with the normal curve for the same dosage of glucose, variable results are obtained. In the penta-methylene series the first two curves (I, IIa) rise to 125 mg. glucose per 100 cc., a value which would be found for 2.0 grams of glucose per kilo. in the normal animal. In Experiment IIIa 6.0 grams of glucose were given in 6 hours, the highest blood sugar value being 160. This value is also normal. In the synthalin series, in the first three series of experiments the blood sugar curves rise to higher levels than they would

TABLE IV
GLUCOSE TOLERANCE OF THE NORMAL RABBIT

Rabbit No.	Wt. Kg.	Date 1929	Amount of Glucose per Kg.	Sugar in 100 cc. blood								Urine
			gm	Before glucose injection	1 hr.	2 hr.	3 hr.	4 hr.	6 hr.	8 hr.	10 hr.	
U7	2.84	Oct. 2	3	93	148	143	118	121	98			neg.
U8	3.01	Oct. 2	3	98	143	143	123	124	90			"
U11	3.12	Oct. 16	5	87	182	190	208	121	100			"
U12	2.78	Oct. 16	5	80	190	230	220	198	105			"
U11	3.12	Oct. 21	10	95	210	264	286	260	182	133		"
U12	2.84	Oct. 21	10	104	184	218	222	188	163	137		"
U11	3.12	Oct. 28	20	102	344	364	392	408	388	228	133	++++ 4th hr.
U12	3.12	Oct. 28	20	108	264	334	364	412	286	174	182	++++ 4th hr.
U13	3.86	Nov. 11	20	104	350	464	592	668	612	496	472	+++ 4th hr.
U14	3.97	Nov. 11	20	98	326	416	424	384	376	364	264	++++ 4th hr.
U13	4.2	Dec. 12	15	95	182	190	192	174	138	115	106	neg.
U14	4.2	Dec. 12	15	95	234	260	254	220	174	114	87	"

have in normal animals. In Experiment IIIa, the rabbit received 10.0 grams glucose per kilo. per os. At 4.5 hours the blood sugar was 276. This value would be found in a normal curve.

Blood Lactic Acid. Simola (1927) first showed that the blood lactic acid rises following the action of synthalin. Staub (1927) and Bodo and Marks (1928) believe that lactic acid is the most important of the immediate effects of synthalin on preformed carbohydrate. We followed the lactic acid content of the blood in six of our series of experiments and observed a rise in blood lactic acid following the action of the guanidine compound in every case.[1] Following the administration of glucose, the lactic acid values continued to rise and then fall in the case of the controls and the insulin experiments. In the case of the adrenalin experiments the last lactic acid value was higher in each case. Cori and Cori (1929) recently showed that the blood lactic acid of the rabbit rises to a maximum at 2 hours after the subcutaneous injection of 0.2 mg. adrenalin per kilo. In our experiments the blood lactic acid was high at the time the adrenalin was given, as a result of the action of the guanidine compound. The adrenalin in our experiments did not increase the blood lactic acid at the time of the corresponding peak of the control curve, but kept the values up at the time the controls and rabbits receiving insulin were falling. The observation of Cori and Cori that adrenalin raises the blood lactic acid has been confirmed. It should be noted that in Experiment VII of the synthalin series, the blood lactic acid for the animal that had received adrenalin rose to the highest level (as in the other series of experiments) but fell below the terminal value for the animal that had received insulin. In this experiment the blood sugar also dropped appreciably the last hour, indicating that the effect of adrenalin had worn off.

Discussion

In studying the effects of synthalin on metabolism, Baltherwick *et al* (1927) established the blood sugar curve on a glucose tolerance and then repeated the experiment giving synthalin after the glucose. His results

[1] It should be noted that our resting blood lactic acid values are higher than those of Cori and Cori, and lower than, or the same as, those of Bodo and Marks. The average resting value of Cori and Cori is 11 mg. per 100 cc. The values of Bodo and Marks range from 80 to 110 mg. per 100 cc. Our values are from 25 to 119 mg. per 100 cc. The high values obtained by us in one series were undoubtedly due to the obstreperous nature of the rabbits used in that series. It should be added, however, that following the action of the guanidine compounds, the rabbits were in a drowsy condition, and offered no resistance to manipulation, so that these blood lactic acid values were not influenced by exercise. The initial blood lactic acid values are of little interest in the interpretation of the data.

were variable. In two of his experiments the blood sugar values are given at the time when the glucose was administered. The rabbit which experienced hyperglycemia when glucose was administered had had a normal blood sugar at the time synthalin was given. The rabbit which utilized or stored all the sugar had had a blood sugar near convulsion level. The variation in results obtained by Blatherwick was due in part to the fact that in some of his rabbits the glycogen reserves of the liver were depleted, while in others considerable glycogen still remained. In several of the synthalin experiments of Bodo and Marks a hyperglycemia preceded hypoglycemia. In studying the action of diguanidine-penta-methylene and octa-methylene, we gave glucose at the time of hypoglycemia and obtained variable results. In some instances all the glucose was used up, in others a hyperglycemia blood sugar curve was obtained. In our present series of experiments the blood sugar curves were variable, as has been pointed out.

In the light of the recent researches in carbohydrate metabolism, it would appear that the determination of the so-called glucose tolerance, with and without the simultaneous action of the guanidine compound, is no criterion as to whether or not the guanidine compound causes utilization of glucose (in the sense that insulin stimulates glucose disappearance). The method could be used if the endogenous supply of glucose were identical under both conditions. We now know that it is not. Prior to guanidine hypoglycemia the liver glycogen is breaking down, in part at least, to form glucose. At hypoglycemia the liver glycogen is depleted and the liver is unable to store glycogen or to produce glucose either by deaminization or by conversion of lactic acid to glucose; the muscle glycogen is greatly reduced or depleted. We therefore feel that neither Blatherwick's nor our own experiments have demonstrated that guanidine derivatives are able to stimulate glucose utilization. Staub (1927) has shown that in the eviscerated preparation the glucose is converted to lactic acid, a process not characteristic of insulin action.

In the normal rabbit the amount of adrenalin given in our experiments would produce alone (that is, without giving glucose simultaneously) a profound hyperglycemia. Following guanidine hypoglycemia, adrenalin alone is unable to produce a hyperglycemia, as was demonstrated by Bodo and Marks and ourselves. The fact that the adrenalin in our present experiments brought about an increase in the blood sugar curve throws some light upon the mechanism of normal adrenalin hyperglycemia and is in harmony with the work of Cori and Cori (1929). It shows that adrenalin hyperglycemia is a result of the diminished use of blood sugar

by the muscles. In the normal animal the liver continues to supply the blood with glucose at the same or increased rate, so that a hyperglycemia results. In the guanidine treated animal, the liver can no longer supply glucose from glycogen by deaminization, or from lactic acid through glycogenesis, so that there is no opportunity for glucose to accumulate in the blood, and adrenalin fails to produce hyperglycemia. However, by supplying the blood from the outside, by injection or administration of glucose, an effect similar to that which takes place in the normal animal may be demonstrated.

Cori and Cori (1929) showed that adrenalin increases the blood lactic acid and by the arterio-venous blood lactic acid difference showed that the muscles are the source of lactic acid. Choi (1928) did not find that adrenalin stimulates the breakdown of muscle glycogen. In contrast, Cori and Cori (1929) hold that the mobilizing action of adrenalin on muscle glycogen is also connected with the increase of blood lactic acid. Cannon (Cori and Cori, 1929) is unwilling to accept the fact that mobilization of muscle glycogen occurs normally and is responsible for the increase in blood lactic acid.

In all of our experiments the muscle glycogen is lowest (in most of them *nil*) for the animals that had received adrenalin. Since control animals killed at guanidine hypoglycemia showed only slightly higher muscle glycogen values than those in our adrenalin series, our glycogen analyses cannot be used in deciding whether adrenalin mobilized muscle glycogen. We found that the blood lactic acid was highest for the animals that had received adrenalin. However, in several series of experiments the muscle glycogen, both of the animals that had received adrenalin, and of the controls, was *nil*, so that one has the choice of three possible explanations. 1.—Muscle glycogen was broken down to lactic acid as fast as it was formed. 2.—The blood lactic acid due to adrenalin activity in the muscle was derived from sources other than glycogen. 3.—Adrenalin decreased the utilization of lactic acid by the muscles. It is entirely possible that all the blood lactic acid produced in our experiments was due to the guanidine compound. In that case, we are forced to assume that adrenalin decreased its utilization. Our experiments show rather conclusively that insulin does not affect the lactic acid.

MacCleod is inclined to believe that "glucose passes through a glycogen stage in the muscles themselves before it is actually used, a view which receives some support in the fact that glycogen is formed in the muscles when glucose is injected into eviscerated cats along with insulin (Best, Hoet, and Marks, 1926), to a less extent, in intact animals without the

injection of insulin (Choi, 1928)". In the series of experiments referred to by MacCleod it was shown that injection of glucose into depancreatized spinal cats, and also in eviscerated preparations, causes no glycogen formation in the muscles, so that insulin must be the direct cause of the storage of the glycogen. In several of our experiments the glycogen content of the muscle was measurable after the administration of insulin, while the control animal showed no glycogen. These experiments show that the endogenous supply of insulin was not sufficient to store glycogen, as it would under normal conditions. In still other experiments, large doses of insulin were unable to store muscle glycogen. In Table V, the glucose

TABLE V

RELATION BETWEEN UTILIZATION OF GLUCOSE AND STORAGE OF GLYCOGEN FOLLOWING SYNTHALIN DEGLYCOGENATION

Experiment	Blood sugar level mg. per 100 cc.	Total glucose given	Glucose recovered	Glucose utilized other than glycogen formation	Glycogen content
		gm.	gm.	gm.	gm.
Control (Va)	620–550	7.5	3.6	3.9	nil
Insulin (Vb)	650– 75	7.5	0.6	6.9	0.8
Control (VIa)	240–210	10.5	1.1	9.4	nil
Insulin (VIb)	260–130	15.7	0.5	15.2	0.2
Adrenalin (VIc)	350–520	10.5	3.8	6.7	0.2
Control (VIIa)	270–160	5.4	0.4	5.0	0.1
Insulin (VIIb)	230–180	18.9	0.4	18.5	1.4
Adrenalin (VIIc)	420–250	5.4	0.6	4.8	nil

The calculations required to compute the data presented are based upon the following assumptions. The "active volume" of a 3.0 kilo. rabbit is taken as 640 cc. The peripheral tissue (muscle) is taken as 50% the weight of the animal. It is assumed that the injected glucose is completely distributed.

stored as glycogen, the glucose not utilized (accounted for in the blood and urine), and the glucose utilized but not stored as glycogen, have been calculated for three series of experiments. In these experiments the glycogen for the control and adrenalin experiment was practically nil, so that the glycogen in the insulin experiment represents the storing of glycogen by insulin. In experiment VI, the insulin stored only a trace of glycogen, if any. In the earlier experiments the glucose could not be calculated, because of the uncertainty of the extent of absorption from the alimentary canal. The blood sugar curves and glycogen analyses run parallel with the data of the experiments presented in Table V. The results show that insulin increases the utilization of glucose with or without the

measurable storage of glycogen at hypoglycemic, normal, or hyperglycemic levels. Since we have been unable to show that insulin will decrease the muscle glycogen at a normal or hyperglycemic level, with the simultaneous glycogen-destroying action of guanidino-poly-methylene, we have given no support to the theory that insulin brings about glucose utilization without the intermediate formation of glycogen. The observation that insulin increases the utilization of glucose in virtually deglycogenated rabbits without storing glycogen can be accounted for on the basis that the glycogen is being utilized as fast as it is formed. The lowered glucose tolerance, observed in several of the control experiments, could be accounted for by a decreased rate of glycogen formation, especially since there was no glycogen storage by endogenous insulin.

In these studies we have shown that certain reactions and changes may be induced to take place *in vivo*, but the question will be raised to what extent they are normal physiological processes. Cannon has recently sounded a warning as to pharmocological doses and physiological deductions. In common with the majority of the recent workers in carbohydrate metabolism, we have been forced to use excessive amounts of hormones in order that the induced changes would be greater than the possible limits of normal variation. Any deductions that are made are based upon the assumption that the experiment in question was an exaggeration of a physiological process.

Our results fit in with the following explanation of carbohydrate metabolism.

The liver is the source of endogenous blood sugar. It does not change injected glucose to a form available for muscle utilization. Insulin increases the utilization and storage of blood sugar by the muscles. The increased utilization of blood sugar by the muscles is dependent upon the intermediate formation of glycogen. Insulin is not concerned in the breakdown in exercise of muscle glycogen to lactic acid with subsequent oxidation of lactic acid.[2] Adrenalin decreases the utilization or storage of blood sugar by the muscles and either increases the lactic acid production of the muscles or decreases the utilization of lactic acid by the muscles.

Summary

1.—By means of the diguanidino-polymethylenes it is possible to deplete the glycogen reserves of the liver and destroy the power of this organ to

[2] It is now a well known observation that the insulin requirement of a diabetic on a known diet is decreased when exercise is taken. This observation is readily accounted for on the basis that the utilization of carbohydrate through the formation of lactic acid is not connected with insulin activity. If insulin were required to oxidize lactic acid, more insulin would be required during exercise.

store glycogen, without destroying the power of the muscles to utilize glucose either by its disappearance or storage as glycogen. It is also possible to deplete the glycogen reserves and prevent the storing of glycogen at a hyperglycemic level for both liver and muscle, without destroying the power to utilize glucose.

2.—Insulin increases the utilization of glucose at normal or hyperglycemic levels in guanidine deglycogenated rabbits with or without storing glycogen.

3.—Adrenalin is able to decrease the utilization of injected glucose in animals in which the liver has been depleted of glycogen and has lost the power of storing glycogen through guanidine derivatives, showing that normal adrenalin hyperglycemia is not entirely due to an increased output of glucose by the liver.

4.—Adrenalin increases the blood lactic acid and insulin is without effect upon the blood lactic acid of rabbits suffering guanidine deglycogenation.

5.—Adrenalin increases the blood lactic acid in rabbits whose liver and muscle glycogen reserves have been depleted (less than .01 per cent) by guanidine derivatives.

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Editorial Review

RECENT ADVANCES IN THE STUDY OF BASAL METABOLISM*

PART I

THE study of basal metabolism is a good example of the results that can be obtained by cooperation between physiologists and clinicians. As a matter of fact the men who first developed this field were trained in both branches of science. At the present time the clinicians are deriving great practical benefits from the tests. They are being made as a matter of routine on all cases suspected of having any disease of the thyroid gland and in practically all cases where the diagnosis is uncertain. Machines for making tests are manufactured in the thousands every year and are to be found in almost every clinic. The result has been an enormous number of experiments, most of them with poor technique. This has led to a corresponding number of publications and the literature of the subject is voluminous. I shall try to cover the more important contributions of the last four years but shall be obliged to leave out all the work that deals with animals and confine myself to a relatively small number of the papers that deal with man.

If we view these last four years as a whole we find that they have not made any great changes in our conceptions of the basal metabolism. We still consider it to be the heat production of an individual measured during complete mental and physical repose, fourteen or more hours after the last meal. The basal metabolism of a given individual is fairly constant and it can be compared with the average normal figure for persons of the same age, sex and size. The one great clinical application is that the basal metabolism is much higher than normal if the thyroid gland is too active, and distinctly lower than normal when the thyroid secretion is deficient.

The technique of basal metabolism determinations was simplified about ten years ago and there have been no striking changes recently. F. G. Benedict (1) has devised a very small portable apparatus for use on exploring expeditions and this is being carried to all sorts of remote places. In Europe there have been a large number of modifications of the closed circuit apparatus designed to measure the CO_2 production as well as the oxygen. The technique is simple, perhaps too simple. These machines are extensively employed on the Continent but not in America and I

* Revised from an address given at the Marine Biological Laboratory, Woods Hole, Mass. on August 5, 1930. *Collecting Net*, Woods Hole, 1930, V, 197.

have not had the opportunity of working with them. One gets the impression that the simpler machines which measure the carbon dioxide production by various short-cuts are not quite reliable enough for research, and for clinical testing offer no great advantage over the closed circuit apparatus which measures oxygen alone. The accurate measurement of the respiratory quotient (volume of CO_2 divided by the volume of O_2) is a delicate bit of technique especially if short experimental periods are employed. There are many factors which may cause the quotient as measured in the apparatus to differ considerably from the true quotient which represents the foodstuffs metabolized in the tissues. These have been fully discussed by Richardson (2) in his comprehensive review of the literature of the respiratory quotient.

The accuracy of an apparatus in determining the quotient can be tested by means of alcohol checks or by placing experimental subjects on diets very low in carbohydrates and producing thus a mild ketosis. Under these conditions McClellan, Spencer, Falk and DuBois (3) using the Sage respiration calorimeter found that all the basal quotients and even the quotients after meals fell between the limits of 0.72 and 0.78. These were the averages of three-hour experiments and probably represent quite accurately the nature of the foodstuffs oxidized. Short period experiments would undoubtedly show a wider range.

Benedict and Root (4) have devised a method of estimating roughly the total metabolism from the insensible perspiration. The subject is weighed on accurate silk scales before going to bed at night and again on arising, or else the loss of weight is measured during a short period in the daytime using a large and extremely delicate balance. The loss of weight is chiefly due to the vaporization of water from the skin and lungs, approximately 85 per cent being accounted for in this manner. The remainder of the loss is caused by the fact that the weight of CO_2 eliminated is greater than the weight of O_2 absorbed except for unusually low respiratory quotients. A great many precautions must be employed in using this method but it gives promise of being of considerable help in the study of metabolism not only in health but also in disease. Soderstrom and DuBois (5) had shown in 1917 that normal men of different ages and patients with many different diseases, when studied under standard conditions, lose about 24 per cent of their calories through the vaporization of water. Levine and Wilson (6) observed an average loss of 26 to 27 per cent of calories through vaporization in normal and marantic infants and confirmed for children the statement that the measurement of the insensible perspiration is a time-saving method of estimating the metabolism.

Normal Standards

The so-called normal standards of basal metabolism have come in for a great deal of well deserved criticism. Many of us realized from the start that there never would be any normal standard that would remain satisfactory for many years. Thus the Sage Standards were put out in a tentative form in 1917 as a footnote to an article by Aub and DuBois (7). These were based on calories per square meter of body surface per hour. A short time later Harris and Benedict (8), using a larger and better series of normals, proposed their well-known prediction formula. Their averages run about 3–4 per cent lower than the Sage figures and on the whole give better agreement with the groups of normal controls that are studied in various laboratories. More and more cases, however, are being reported that average even lower than the Harris-Benedict standards. Benedict (9) himself, in a second series of 27 normal men and 33 normal women, finds that the men average 1.8 per cent below the Harris-Benedict prediction figures and 4.4 per cent below the Sage standards. The women averaged 4.2 per cent below the Harris-Benedict and 7.3 per cent below the Sage. He advocates lowering the standards for women by 5 per cent believing that their metabolism is about 12 per cent below that of men instead of only 7 per cent. There is one notable exception to the downward trend of metabolism and that is found in the large series of 1822 males and 5066 females reported by Boothby and Sandiford (10) at the International Congress of Physiology in Boston. This series from the Mayo Clinic corresponds very closely with the Sage standard of 1917, being higher for some of the age periods but 1 or 2 per cent lower for most of the decades. Here we have a very large series of normal controls studied with excellent technique. Why so much difference in the levels between Boothby's controls and the controls in physiological laboratories?

The answer is probably to be found in the fact that the Sage standards and those of Boothby and Sandiford were intended to serve primarily as the basis of comparison for patients studied for clinical purposes. Both series contained a high proportion of first tests and the early Sage group included tests made in the days when the precautions about absolute mental and physical rest were not observed as scrupulously as at the present time. The Harris-Benedict group contained relatively few first-day tests and many observations on trained subjects. In the last few years for all extremely careful testing, subjects are selected and their tests are not counted until they can assume the complete relaxation that ensures the minimal metabolism. Almost all trained subjects who have had many metabolism tests give results well below all the normal standards and

their metabolism remains very constant, falling gradually as they grow older.

On the other hand, it has long been known that athletes and men accustomed to hard labor have a higher metabolism than others. Thus Takahira (11) found that general laborers averaged 5.8 per cent above the mean, policemen 3.3 per cent above, the school teachers and tradesmen 3 to 7 per cent below the mean.

These two factors of 1, experience in metabolism tests with complete relaxation and 2, physical condition, are of great importance in interpreting the results of tests on individuals or on groups of persons. Thus if a man wanted to prove that the metabolism of the inhabitants of some city was lower than that of the country in general, he could select a group of non-athletic persons, train them in the art of complete relaxation until they approached "cadaveric repose" as the Germans call it. Then if the results of the first three or four days were discarded and the lowest test out of three made during the morning were selected, he would find that his average was 5 or 10 per cent below the so-called normal standards. It has been argued that one should select the lowest test as representing the true basal because there are practically no factors except sleep, undernutrition and disease that lower the metabolism below the basal, but many experimental errors that raise it. This argument does not seem to me quite fair as there is an experimental error of at least two per cent in any test due to the apparatus and the technique. Personally, I prefer to make three or four short tests and take the average of the two lowest that agree fairy closely.

Now if another experimenter wished to prove that the metabolism was high, he could select a group of laborers and athletes, make tests on one or two days only, include all the days, take the average of the three tests made each day, and he could easily show a level of heat production well above the average for the country at large.

This may sound extreme but it is just what is being done in all parts of the globe as we shall see when we come to the discussion of the metabolism of different races. Many of these reports are filled with elaborate statistical discussions and analyses. One gets the impression that some investigators try to compensate for a poor selection of material by an impressive statistical treatment. Perhaps this impression of mine is merely the personal reaction of a clinician who is not well trained in statistics. All clinicians are suspicious of statistics. They realize the difficulty of diagnosis, the frequency of incipient disease among groups of so-called normal subjects, the wide variations found in humans, the large number

of factors that may affect experiments. If any one individual shows a large departure from the mean of the group, the statistician wants to magnify the importance of this result; the clinician is inclined to throw the result out of the averages entirely.

Among the many factors that may affect metabolism we are often given very little information about the diet. A liberal diet tends to raise the metabolism even fourteen hours after the last meal, a low diet leads to a lowered metabolism. The effect of varying the protein ration within moderate limits seems to be minimal. We have recently had in our laboratory the opportunity of studying in considerable detail the metabolism of two arctic explorers, Stefansson and Andersen, who lived for one year on an exclusive meat diet (12). They ate no carbohydrate except the 5 or 10 grams contained in the meat; they consumed daily 100 to 140 grams of protein and 200 to 300 grams of fat. This moderate increase in the protein ration caused no change in the basal metabolism. Wang (13) and her associates have recently reported on a series of six young women who tried first a diet of 2 grams of protein per kilogram of body weight and then a diet of 0.6 grams of protein per kilo. Even though each experimental period was continued for about a month, there was no change in the basal metabolism. The matter is not completely settled, but it looks as if we could neglect minor changes in the protein ration.

Metabolism of Women

It has long been known that the metabolism of women is more variable than that of men. In the last ten years we have come to realize that part of this variability is due to the effect of the menstrual cycle. I think we can say that it is only in the last five years that this effect has been definitely proved. It is not great, it is not present in all women, and the maxima and minima do not always come at the same phases of the cycle. Benedict and Finn (14) have reported on the artists' model, Miss W., who has been studied at the Nutrition Laboratory in Boston for the last twelve years. She is an ideal subject, phlegmatic in disposition, never ill and never suffering any discomfort at the menstrual periods. During these twelve years the average metabolism of the menstrual days was 3.5 per cent lower than the average of the non-menstrual days. There was a distinct premenstrual rise in heat production and also an intermenstrual peak as had been reported in the earlier literature. Hitchcock and Wardwell (15) made 800 tests on 27 women and selected finally the 20 best ones as the basis of their report. Of these 20 women 14 showed a lowering of the basal metabolism during the menstrual period, one had no change and five showed a

slight but insignificant rise. For the group of ten women whose fall in metabolism was of statistical importance, the average drop was 5.16 per cent. There was also an intermenstrual drop averaging 5.03 per cent occurring on the 12 to 23rd day, on the average the 15th day. Since ovulation occurs on the 14 to 28th day following the onset of menstruation, Hitchcock and Wardwell believe that there is some relationship between the two phenomena. These authors incidentally report the effects of emotion on these women. One student before her Ph.D. examination averaged 40.12 calories per square meter per hour, and almost immediately after the examination came to a level 10 per cent lower. Another young medical student spent her first exciting day in the dispensary and on the next morning the metabolism had risen from its usual level of 32 calories per square meter to 42 calories. Parties on the evening before the tests lowered metabolism.

Griffith, Pucher, Brownell, Klein and Carmer (16) in their admirably detailed report, confirm in general these findings, observing the highest metabolism in the premenstrual period and the lowest during the menstrual days. The average difference is not great, only 2.7 per cent. They confirmed the older observation that pain raised the heat production and excluded all cases where this was a factor. Conklin and McClendon (17), in a recent study of ten women who suffered no menstrual pain, found that in nine of them the metabolism tended to reach its lowest level following menstruation and its highest level preceding menstruation. The average difference was only 3.5 per cent.

In recent years it has been the custom to tell women not to have the clinical metabolism tests made during the menstrual period. It would seem even more important to avoid the premenstrual week, which gives results higher than the average. This would leave only the two intermenstrual weeks, but there is a good deal of evidence for a second peak and second valley in this fortnight and their exact times are not predictable. Why, then, should we try to avoid the one phase when the metabolism is uniformly low? It would seem more practical to note the time when the tests happen to fall in the menstrual cycle and take this into consideration in the interpretation of the results. The clinician who does not take a good many factors into consideration when he interprets his results has no business to ask for metabolism tests.

Racial and Climatic Factors

Now is it any wonder that investigators have reported differences of metabolism in different races? There is a very pretty little polemic waging

in Dutch, French and Spanish, and I may have missed some of the fine points, but the gist of the matter is as follows: Way back in 1896 Eijkmann (18) (Eykman) working in Batavia and Holland reported that the metabolism of the Malays was the same as that of the Europeans living in Java and that the Europeans studied in Java and in Holland showed no significant change in metabolism. Ozario de Almeida (19) in Brazil in 1920 reported figures for whites in the tropics 24 per cent below the metabolism of North Americans, and the negro laborers only 8 per cent higher than the whites. He inferred that Eykman's technique was old-fashioned and Teding van Berkhout (20) reporting low metabolism in Java supported Almeida's criticism. Eykman (18, 21) retorted vigorously, proved that his work had been done with every modern precaution and pointed out that when Almeida (22) repeated his work he found a higher metabolism than in his original report. Meanwhile Montoro (23) in Cuba had announced a series of tests in Havana that averaged 13 to 15 per cent below the Sage and Harris-Benedict standards. Coro (24), also of Havana, disagrees with Almeida and Montoro and reports on a series of student nurses with an average exactly the same as the Sage standards for North America. Mazzocco (25) working in the Argentine Province of Salta made a careful study of 76 persons, most of them artillery conscripts 20 to 21 years of age. He found their metabolism 39.5 calories per square meter per hours, which is just the same figure as the so-called Aub and DuBois Sage standard. Ninety-three and two-tenths per cent of his men were within 15 per cent of the standard.

There are two reports that indicate a slightly lowered metabolism of young women in our Gulf States. Hafkesbring and Borgstrom (26) report that the average for their 9 subjects in New Orleans was 16 per cent below the Harris-Benedict standard and 18 per cent below the Sage. Tilt (27) studying a large series of young college women in Florida between the ages of 17 and 25 obtained averages 9.9 per cent below the Harris-Benedict and 10.6 per cent below the Aub-DuBois standard. Tilt's contribution is particularly valuable because she had participated in a study of the metabolism of girls in the northern part of the United States (28).

Perhaps the most significant piece of work is that of MacLeod, Crofts and Benedict (29) who found that a group of seven Chinese and two Japanese women who had been students in America for more than a year were 10 per cent below both the Sage and the Harris-Benedict standards. Even if these standards are both 5 per cent too high, this leaves the Oriental group 5 per cent below their fellow students. Takahira (11) has

shown that the average metabolism of a series of 120 Japanese men and women is 5 to 7 per cent below the Sage standards, but, as I have said before, these standards may be 5 to 6 per cent too high for a well trained group, so that Takahira's work indicates no significant racial difference. Okada, Sakurai and Kameda (30) in a series of Japanese students and nurses obtained figures close to the Sage standards. Earle (31), on the other hand, came to the conclusion that his large series of 166 Chinese and 15 other Orientals indicated a slightly lower metabolism than that of Westerners. His Chinese averaged 8 per cent below the Sage, Dreyer and Harris-Benedict standards; his other Orientals and 41 Westerners living in Hong-Kong were about 7 per cent below. Stevenson (32) has done some work on the height-weight surface area used in these calculations which indicates that it may not apply accurately to the Chinese and this may possibly account for some of the apparently lowered figures based on calories per square meter per hour, but his work has not convinced Earle.

Necheles (33) is inclined to ascribe the lower metabolism found by Earle to the greater relaxation of the Chinese. He himself made a few observations on Chinese and Westerners awake and asleep. With the Chinese the fall in heat production caused by sleep averaged about 2.6 per cent, whereas with one Westerner it was 14 per cent and in another 27 per cent. These last figures are much higher than those observed by most experimenters. It is customary in this country to estimate that profound sleep causes a fall of about 10 per cent below the so-called basal. The suggestion of Necheles is well worth considering though not yet established.

Sundstroem (34) observed a metabolism in North Queensland, Australia, about as low as that found by Almeida. The average for men was 31.0 calories per square meter per hour and for women 27.8 calories. Hindmarsh (35) found that white students in Australia averaged 9 per cent below the Sage standards. Wardlaw and Horsley (36) working in a slightly warmer part of Australia studied a series of eight aborigenes living on a government reserve where they received ample rations once a week in return for a very small amount of labor. They lived wastefully and the brief period of plenty was followed by a period of want for the rest of the week. Most of their time was spent in lounging and sleeping. As might be expected, their metabolism was low but still the average of 30.7 per cent below the Sage standard is very striking. Here in America it takes a great deal of undernutrition to depress the basal metabolism to such a level. It is possible that the low level of these blacks might have been entirely due to the fact that they were indolent and underweight. One half-caste, not

included in the series, was a hard worker and muscular. His metabolism was slightly above the Sage standard.

Steggerda and Benedict (37) found in Jamaica that a series of eight full-blooded blacks and five brown women gave results very close to the Harris-Benedict standard but that the 37 male browns averaged 5.4 per cent low. They did not consider this small difference to be of significance. Williams and Benedict (38), in a study of the workers on an archeological expedition to Yucatan, observed no appreciable change in the metabolism of the whites after they had lived for a short time in this climate. The 32 male Mayas who acted as laborers in the excavations gave an average 5.2 per cent above the Harris-Benedict standards and Benedict, although he admits the possible effect of the hard labor, is inclined to believe that some of this difference is racial. Turner and Abonshadid (39) have reported that the basal metabolism of a series of 28 young Syrian women averaged 13.3 per cent below the Sage standards and that the American and European women living in Syria averaged 6 per cent below. The Syrian women lived a quiet life with little manual labor and this could easily account for their somewhat low metabolism.

Benedict (38) has noticed that the scientists of some of the races that have shown a slightly lowered metabolism have acted as if this implied some degree of racial inferiority. He has hastened to point out that the highest metabolism has been found among the Mayas who have not contributed much to civilization for many centuries. As a matter of fact, a race might point to a lowered metabolism with pride as indicating a greater mental repose or a greater economy of physiological effort. After all, one gets the impression that the racial differences are so slight that they are almost entirely obscured by the factors of repose, physical training and nutrition.

Obesity

In the last few years there has been a great deal of work on the metabolism in obesity and apparently most of this work has been wrong. We have not added much to our total knowledge since 1915 when Means (40) in Boston demonstrated that the metabolism of the obese was normal when calculated according to surface area. Physicians and laymen also have been puzzled by the fact that some obese persons are apparently light eaters and that some will not lose weight even when placed for two or three weeks on diets that are obviously too low in calories to meet the caloric expenditure. In order to explain these phenomena they have suggested that the basal metabolism is low or that work is carried on more economically. This is not true. They have reported in a large series of

papers in Germany, Austria and America that the specific dynamic action of food is abnormally low in obesity and have drawn a good many curves that would seem to substantiate this. The specific dynamic action is the stimulation in metabolism that follows the ingestion of food and a good sized protein or carbohydrate meal may raise the heat production 20 to 40 per cent for a few hours. Now the curves for specific dynamic action are hard to measure and are affected by a great many factors, particularly if short test periods are employed. They are even worse than glucose tolerance curves. It is small wonder that the results are conflicting. Spencer and McClellan (41) using all the precuations of the Sage metabolism ward and the Sage calorimeter, have studied a fairly large series of obese men and women and have obtained normal curves for the specific dynamic action. They have found no abnormality of metabolism except that the older obese patients have less ketosis on low carbohydrate diets than the normal controls. Even the depression of metabolism in sleep is no greater for the obese than for the normals.

Quite a few writers on the subject of obesity have gone so far as to suggest theories that violate the law of the conservation of energy. The chief evidence by means of which they would upset this law is the statement of certain fat people that they are light eaters. These people are supposed to be suffering from some form of endocrine disorder and they are classed under the heading of "Constitutional or Endogenous Obesity."

For a good many years we have known that when diets are changed the variations in weight are chiefly due to the retention or loss of water loosely bound in the tissues of the body. It has been believed that a fat person suddenly placed on a diet which supplied only half the calories expended, retains enough extra water in his tissues to mask the inevitable loss of body fat for one, two or even more weeks. This phenomenon has been admirably discussed in a recent report by Newburgh and Johnston (42–44) of Ann Arbor. With infinite labor and care they determined the water balance of a series of fat persons. They calculated the total heat production by means of tests of the respiratory metabolism and for the 24-hour period supplemented this by using the careful measurement of the insensible perspiration, according to the method of Benedict and Root. If proper precautions are employed, this gives a fair measurement of the total heat production.

Newburgh and Johnston were able to show that their obese patients, when losing body tissue on submaintenance diets, held or even gained weight for considerable periods, the longest being 16 days. They proved that this was due to a retention of water and they noted in all cases a

sudden loss of weight as this water was eliminated with a return of the weight curve to the point that was predicted from the daily loss of body tissue. They emphasize the old conservative belief that obesity is caused by eating more food than is needed.

(*To be continued*)

E. F. D.

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